Acta Medica Hungarica

VOLUME 50, NUMBERS 1–2, 1994

Akadémiai Kiadó, Budapest

ACTA MED. HUNG. 50 (1–2) 1–132 (1994) HU ISSN 0236–5286

ACTA MEDICA HUNGARICA

A QUARTERLY OF THE HUNGARIAN ACADEMY OF SCIENCES

Acta Medica publishes reviews and original papers on clinical and experimental medicine in English. *Acta Medica* is published in yearly volumes of four issues by

AKADÉMIAI KIADÓ
Publishing House of the Hungarian Academy of Sciences
H-1117 Budapest, Prielle K. u. 19–35

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H-1083 Budapest, Szigony u. 43 or H-1450 Budapest 9, P.O. Box 67

Subscription information

Orders should be addressed to

AKADÉMIAI KIADÓ
H-1519 Budapest, P.O. Box 245

Subscription price for Volume 50 (1994) in 4 issues US$ 84.00, including normal postage, airmail delivery US$ 20.00.

"This periodical is included in the document delivery program THE GENUINE ARTICLE of the Institute of Scientific Information, Philadelphia. The articles published in the periodical are available through *The Genuine Article* at the Institute for Scientific Information, 3501 Market Street, Philadelphia PA 19104."

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Acta Medica Hungarica, Vol. 50/1—2, pp. 3—13 (1994)

NEUROENDOCRINOLOGY

HYPOTHALAMIC DYSFUNCTION IN PARKINSON'S DISEASE PATIENTS

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(Received: May 6, 1994)

Ten patients with idiopathic Parkinson's disease (PD) (3 men and 7 women, group A) who had received no treatment for the disease; 102 patients with PD (36 men and 66 women, group B) who had undergone treatment and 45 healthy volunteers (15 men and 30 women, control group) were subject to thyrotropin-releasing hormone (TRH) tests and levodopa tests. In group A basal plasma prolactin (PRL) levels were significantly higher than in the controls both before and during treatment. Peak plasma PRL levels during TRH tests were significantly higher before treatment, but returned to the control levels during treatment. Nadir plasma PRL levels during levodopa tests were significantly increased before and during treatment. In group B basal plasma thyroid-stimulating hormone (TSH) and PRL levels were significantly higher than in the control group. Peak plasma PRL levels during TRH tests and nadir plasma PRL levels during levodopa tests were also significantly increased. The results strongly suggest a disturbance of pituitary hormone secretion due to hypothalamic dysfunction in PD patients.

Keywords: Parkinson's disease, hypothalamus, TRH test, levodopa test

Introduction

Idiopathic Parkinson's disease (PD) is a neuro-degenerative disease. Its principal clinical features are caused by dopaminergic deficits in the nigrostriatal area. Besides, dopaminergic deficits and morphological changes may occur in the hypothalamus /19, 23/, and reduction of noradrenaline /21, 38/, serotonin /15, 38/, substance P /3, 32, 41/ and somatostatin concentrations /1, 10/ in the brain have been reported in PD patients. Dopamine is

Abbreviations: PD: Parkinson's disease; TRH: thyrotropin-releasing hormone; PRL: prolactin; TSH: thyroid-stimulating hormone; GH: growth hormone; SD: standard deviation

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0236-5286/94/$ 4.00

one of the most notable neurotransmitters in the hypothalamus, as it inhibits thyroid-stimulating hormone (TSH) release /7/, prolactin (PRL) release /17/ and stimulates growth hormone (GH) release /6/. Since studies of the secretion of pituitary hormones in PD patients had produced inconsistent findings, we investigated the basal and stimulated plasma hormone levels and the response pattern of TSH, PRL and GH to thyrotropin-releasing hormone (TRH) and levodopa in both untreated and treated PD patients to clarify hypothalamic dysfunction.

Subjects

Ten patients with PD (3 men and 7 women, group A) who had received no treatment were examined twice, the first time before treatment and the second time during each patient's particular levodopa treatment regimen. Clinical diagnosis of PD was determined on the basis of history, symptoms, neurological examination and brain CT. The mean age in group A was 56.2 ± 7.18 years (mean $\pm$ standard deviation /SD/), and the mean Yahr score (an index indicating severity of disease) was 1.3 ± 0.5 (Table I). A hundred and two patients with PD (36 men and 66 women, group B) were examined during each patient's particular levodopa treatment regimen. The mean age of the patients in this group was 62.8 ± 8.24 years, and the mean Yahr score was 2.8 ± 1.0. Forty-five control subjects (15 men and 30 women) were both neurologically and endocrinologically asymptomatic. The mean age of the control subjects was 58.4 ± 4.75 years. Depression was ruled out for all subjects by means of the Hamilton Rating Scale for Depression /25, 37/. Informed consent was obtained from all subjects, and all women in this study were postmenopausal.

Table I

Clinical characteristics of group A

No.	Sex	Age	Severity of disease (Yahr)	Duration from onset (in years)	Total levodopa dose (gram)
1	M	52	I	0.7	25.2
2	F	65	II	1.6	34.5
3	M	63	II	1.2	29.1
4	F	42	I	0.5	18.3
5	M	58	I	0.8	38.4
6	F	61	I	0.9	21.6
7	F	59	I	0.7	28.8
8	F	54	II	1.8	36.9
9	F	48	I	0.6	37.5
10	F	60	I	1.5	32.7

Methods

The following endocrinological tests were performed.

A. TRH test: 500 μg synthetic TRH was administered by intravenous injection.

B. Levodopa test: 500 mg levodopa was administered orally.

All subjects underwent the tests in the morning after fasting overnight. Blood samples were drawn 30 min before administering TRH and levodopa, and at 30 min intervals thereafter for 2 h. Plasma was separated and stored at -20 ^{o}C until assayed. Two tests were conducted not less than 4 days apart. Group A was tested before and during treatment (at 15.2 $\pm$ 2.04 weeks) with a stable dosage of levodopa combined with benserazide. Group B was tested only once during treatment (at 5.66 $\pm$ 4.14 years) with a stable dosage of levodopa combined with benserazide. Drugs for treatment were last taken in the evening before the tests. Delayed response of plasma TSH during TRH tests meant a TSH level less at 30 min than at 60 min /12/. Paradoxical response of plasma GH during TRH tests was defined as differences $>$5 ng/ml /27/. Hyporesponse of plasma GH during levodopa tests was defined as differences $<$ 5 ng/ml /33/. Group B was subdivided according to severity of disease (Yahr), duration from onset (in years) and total levodopa dosage from initial treatment (in grams). The severity of PD was assessed according to Hoehn and Yahr /16/. Plasma TSH, PRL and GH levels were measured by commercially-available radioimmunoassay kits (Dainabot, Tokyo, Japan). Statistical analysis was carried out using the Kruskal-Wallis test. Group data are presented as the mean $\pm$ SD, and $P < 0.05$ was considered to represent significant difference.

Results

Basal plasma TSH, PRL and GH levels

In group A the basal plasma TSH levels were slightly higher than those of the control group, while the basal plasma GH levels did not differ appreciably from the control values. However, the basal plasma PRL levels were significantly increased before treatment. Elevated plasma PRL levels remained high during treatment. In group B the basal plasma TSH and PRL levels were significantly higher than those of the controls (Table II).

TRH test

In group A, delayed response of plasma TSH during TRH tests was found in 40% of the patients before treatment and in 10% during treatment. Peak plasma PRL levels during TRH tests were significantly higher before treatment but returned to the control levels during treatment (Fig. 1). Paradoxical response of plasma GH during TRH tests was recognized in 40% of the patients before treatment and in 20% during treatment. In group B delayed response of plasma TSH during TRH tests was found in 28%. Peak plasma PRL levels during TRH tests were significantly higher than those of the controls.

Table II

Basal and stimulated plasma hormone levels and response patterns of groups A, B and controls

			Controls (n=45)	Group A (before treatment, n=10)	Group A (during treatment, n=10)	Group B (n=102)
TRH test	TSH (μU/ml)	basal	1.15 ± 0.48	1.93 ± 1.26	1.2 ± 0.77	3.14 ± 1.49[a]
		peak	10.8 ± 2.29	9.92 ± 4.2	9.47 ± 3.58	11.6 ± 5.42
	PRL (ng/ml)	basal	8.53 ± 3.18	21.8 ± 7.85[a]	17.3 ± 9.2[a]	18.2 ± 5.19[a]
		peak	64.4 ± 13.1	109 ± 33.2[a]	66.5 ± 25.4[b]	81.5 ± 30.4[a]
	GH (ng/ml)	basal	1.17 ± 0.6	0.96 ± 0.81	1.16 ± 0.73	0.91 ± 0.62
		peak	1.84 ± 0.68	4.25 ± 3.46	3.82 ± 3.19	4.8 ± 3.27[a]
Levodopa test	PRL (ng/ml)	basal	9.22 ± 2.58	22.5 ± 5.07[a]	18.5 ± 5.54[a]	20.5 ± 4.65[a]
		nadir	3.62 ± 1.11	10.4 ± 2.38[a]	9.68 ± 3.72[a]	9.65 ± 2.23[a]
	GH (ng/ml)	basal	1.25 ± 0.56	1.02 ± 0.52	0.96 ± 0.67	1.06 ± 0.79
		peak	9.26 ± 2.56	6.62 ± 3.64	8.09 ± 3.01	9.64 ± 5.63
TRH test	delayed response of TSH		0%	40%	10%	28%
	paradoxical response of GH		0%	40%	20%	25%
Levodopa test	hyporesponse of GH		0%	60%	40%	38%

Data are expressed as mean ± SD; [a]$P < 0.05$: significant difference between controls and groups A or B; [b]$P < 0.05$: significant difference between group A before treatment and group A during treatment

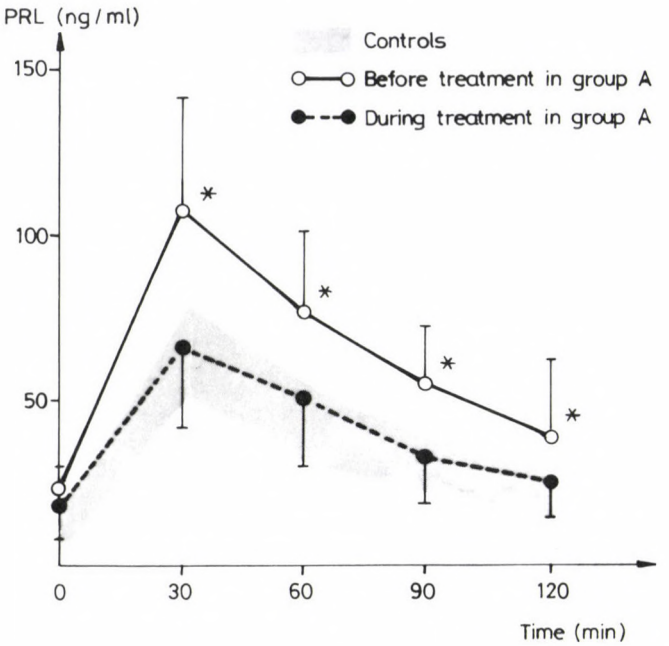

Fig. 1. Effect of TRH on plasma PRL levels in group A and controls. Data are expressed as mean ± SD; *significant difference between group A before treatment and group A during treatment; $P < 0.05$ was considered to represent significant difference

Paradoxical response of plasma GH during TRH tests was recognized in 25% of the patients (Table II).

Levodopa test

In group A nadir plasma PRL levels during levodopa tests were significantly higher both before and during treatment. Hyporesponse of plasma GH during levodopa tests was recognized in 60% of the patients before treatment and in 40% during treatment. In group B nadir plasma PRL levels during levodopa tests were significantly higher. Hyporesponse of plasma GH during levodopa tests was recognized in 38% of the patients (Table II).

Correlation between clinical findings and plasma hormone levels

No correlation was found between severity of disease (Yahr), duration from onset (in years) or total levodopa dosage from initial treatment (in

grams) vs. the following: basal TSH levels; basal PRL levels; basal GH levels; peak TSH levels during TRH tests; peak PRL levels during TRH tests; peak GH levels during TRH tests; nadir PRL levels during levodopa tests; and peak GH levels during levodopa tests.

Discussion

We found that in group A basal plasma TSH levels before treatment were slightly elevated, whereas peak plasma TSH levels during TRH tests were normal. Delayed response of plasma TSH during TRH tests was recognized in 40% of the patients before treatment in group A. It has been reported /4, 13, 31/ that, in untreated PD patients, basal plasma TSH levels are normal, and peak plasma TSH levels during TRH tests are either normal or lower than those of controls. Our findings corresponded with these reports regarding basal plasma TSH levels and peak plasma TSH levels during TRH tests, but when analyzing the response pattern of plasma TSH during TRH tests, we often found delayed responses in PD patients. We have failed to find such observation mentioned in previous report.

It is widely known that a slight elevation in basal plasma TSH levels /36/ and delayed response of plasma TSH during TRH tests indicate the existence of hypothalamic lesion, and that this delayed response exhibits peak levels between 60 min and 120 min /12, 39/. The present data suggest that decreases in TRH secretion may exist in PD patients, although the mechanism for this phenomenon remains unclear.

As mentioned above, dopamine, noradrenaline, serotonin, substance P and somatostatin concentrations in the brain are lowered in PD patients. Reportedly, dopamine either inhibits /20/ or stimulates the secretion of TRH /18, 24, 28/; therefore, its effects on TRH secretion remain controversial. However, the secretion of TRH is stimulated by noradrenaline or serotonin /9, 22/, inhibited by substance P or somatostatin /34, 40/, and regulated by many other neurotransmitters. Accordingly, it should be considered that complex changes in concentrations of many neurotransmitters may be responsible for any reductions in TRH secretion in PD patients.

We found that in group A basal plasma PRL levels and peak plasma PRL levels during TRH tests were significantly elevated before treatment. It has been reported /2, 8, 42/ that, in untreated PD patients, basal plasma PRL levels are either normal or higher than in the controls, and that peak

plasma PRL levels during TRH tests are normal or lower than the control values. Our findings did not coincide with those in other reports regarding basal plasma PRL levels and peak plasma PRL levels during TRH tests. The reason for these differences is unclear. Perhaps, the other studies included too few controls or the controls were not properly matched to PD patients by age and/or sex. It should be noted that PRL levels can be influenced by age and/or sex. The differences may also be attributed to the differences in the conditions of patients and controls.

It is widely known that PRL levels are primarily regulated by inhibiting factors such as dopamine, and that the dopamine concentration in the hypothalamus decreases in PD patients. If so, elevations in basal plasma PRL levels and enhanced PRL response during TRH tests may result either from deficiencies in PRL-inhibiting factor or increases in the sensitivity of lactotroph to TRH. Since the plasma PRL levels remained significantly high during treatment, the present study suggests that, although levodopa therapy greatly alleviated clinical symptoms, levodopa therapy alone may not be sufficient in sustaining these favourable results in view of the reduction of many neurotransmitters in PD patients. It is thus suggested that elevated basal plasma PRL levels and enhanced PRL response during TRH tests result from dopamine deficiency.

We found that in group A basal plasma GH levels were normal; paradoxical response of plasma GH during TRH tests was recognized in 40% of patients; and hyporesponse of plasma GH during levodopa tests was recognized in 60% of patients before treatment.

It has been reported /13, 42/ that, in untreated PD patients, basal plasma GH levels, like the peak plasma GH levels during levodopa tests, are both normal. Our findings were similar to these reports regarding basal plasma GH levels and peak plasma GH levels during levodopa tests. In analyzing the response patterns of plasma GH during TRH tests and levodopa tests, however, we often observed paradoxical response of plasma GH during TRH tests and hyporesponse of plasma GH during levodopa tests. Paradoxical response of plasma GH during TRH tests has been recognized in cases of acromegaly /29/, depression /26/, anorexia nervosa /27/, renal failure /14/, severe liver disease /35/, insulin-dependent diabetes mellitus /5/, hypothalamic tumors and Huntington's chorea /30/. However, PD cases showing this feature were mentioned in none of the cited reports.

The mechanism of paradoxical response of plasma GH during TRH tests is thought to involve either the dysfunction of membrane receptors in GH-

producing cells at the pituitary locus or an alteration in some peptides at the hypothalamic locus /11/. Such alterations at the hypothalamic locus caused by a reduction in dopamine may also be considered. Reduced response of plasma GH during levodopa tests may be a result of the deficiency of GH-releasing hormone. It is well known that GH is stimulated by dopamine, serotonin and arginine, and that elevation of plasma GH induced by dopamine is mediated by stimulating GH-releasing hormone. Moreover, studies on obese and aged subjects have shown decreased peak plasma GH levels during levodopa tests.

In our study, however, reduced response of plasma GH during levodopa tests in group A was unrelated to obesity or age, for the patients were well matched to controls by age and none of the subjects was obese. On the other hand, because dopamine concentration in the hypothalamus decreases in PD patients, it is suggested that reduced response of plasma GH during levodopa tests is a result of this dopamine deficiency. Hyporesponse of plasma GH during levodopa tests was still recognized in 40% of patients during treatment in group A, indicating that hyporesponse of plasma GH during levodopa tests does not completely improve in PD patients during treatment. Some cases which showed abnormal responses during TRH tests or levodopa tests improved during treatment. This means that levodopa treatment alleviated not only clinical symptoms but also hypothalamus-pituitary dysfunction.

In group B, basal plasma TSH levels and peak plasma PRL levels during TRH tests were significantly higher than the control values. This means that hypothalamic dysfunction may again increase in some patients over the course of clinical treatment. The fact that not only dopamine but also several neurotransmitter systems were found to have decreased in the brains of PD patients suggests that the effectiveness long-term supplementary levodopa therapy may be limited in the treatment of PD patients.

Moreover, it is possible that plasma hormone levels were disturbed in the early stages of PD, for we failed to find any correlation in this study between severity of PD and plasma hormone levels.

We conclude that in PD patients the regulatory mechanism for secretion of TSH, PRL and GH is disturbed in the hypothalamus. This dysfunction can be alleviated by treating PD patients with levodopa.

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Acta Medica Hungarica, Vol. 50/1—2, pp. 15—21 (1994)

ENDOCRINOLOGY

THE REACTION OF ADENOHYPOPHYSIS HORMONES IN PRIMARY HYPERPARATHYROIDISM AND AFTER SURGICAL TREATMENT

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(Received: August 30, 1993)

The anterior pituitary hormone serum levels of 13 patients of both sexes were examined for basal secretion and response to TRH-GnRH stimulus before and after parathyroidectomy. The patients were suffering from hyperparathyroidism of parathyroid adenoma origin. After the opoeration, the previously high serum calcium and low serum phosphate levels decreased ($P < 0.001$). The serum parathyroid hormone (PTH) concentration, too, decreased significantly. After surgery, the basal and stimulated secretion of thyrotropin (TSH) showed a significant increase ($P < 0.02$ and $P < 0.05$, respectively). Significantly higher prolactin (PRL) levels were measured after surgery in those patients whose PRL levels were normal both before and after the operation. No significant change was observed in patients with hyperprolactinaemia. After surgery an increased spontaneous growth hormone (GH) secretion was found, while the basal luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretions remained unchanged. In postmenopausal women the stimulated FSH secretion decreased ($P < 0.05$), while the decrease of the stimulated LH secretion was not significant. The results suggest that extracellular calcium may modify the secretion of certain adenohypophysis hormones and their stimulus-induced response.

Keywords: Pituitary hormones, hyperparathyroidism, serum calcium level, effect of surgical treatment

Introduction

The significant role of cytosolic free calcium ion in various cellular activities and, especially in hormone secretion, was extensively investigated and the need for calcium in secretion was proved in several peri-

Abbreviations: FSH: follicle-stimulating hormone, GH: growth hormone, GnRH: gonadotrop releasing hormone, LH: luteinizing hormone, PRL: prolactin, PTH: parathyroid hormone, TRH: thyrotropin releasing hormone, TSH: thyrotropin

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pheral hormones, such as aldosterone or insulin /21, 23/. As far as the pituitary hormones are concerned, intracellular calcium was proved to act as a positive mediator /4, 5, 10, 12, 13, 17/. In recent years animal experiments /19, 20/ and human investigations have demonstrated /1, 2, 6--9, 14, 15, 22/ that extracellular hypercalcaemia can inhibit the release of different pituitary hormones. However, most of the human studies were limited to the investigation of the change in TSH /7--9, 15/, while the results of PRL, FSH, LH and GH release remained contradictory /1, 2, 6, 14, 18, 22/. Our aim was to study in humans the effect of changes in extracellular calcium levels on the basal and stimulated secretion of pituitary hormones. The spontaneous and the releasing factors stimulated secretion of FSH, LH, TSH and PRL were measured before and after surgery.

Patients and Methods

Thirteen patients (6 men and 7 women, aged between 15 and 76 years) with diagnosed hyperparathyroidism induced by parathyroid adenoma were included. Serum total calcium, serum phosphate, PTH concentrations, and GH, FSH, LH, PRL and TSH levels were measured before and after TRH + GnRH administration, pre- and postoperatively. Postoperatively, the investigation was generally performed on the seventh day after the operation. During the test, medications that could influence calcium, phosphate or hormone levels were not given.

As TRH + GnRH stimulus, resting patients were given 0.2 mg TRH (Berlin-Chemie, Berlin, GDR) and 0.1 mg LHRH (Ferring, Kiel, GFR) iv, at 07.00 a.m. Blood samples were taken for measurement of the hormone levels at 0, 15, 30, 60 and 120 min (TSH was measured at 0, 30 and 60 min).

The measurement of GH (RIA, Institute of Nuclear Medicine, Hungarian Academy of Sciences, Budapest, Hungary), FSH, LH TSH (DELFIA, Wallac Oy, Turku, Finland), PRL (RIA, Institute Frederic Joliot-Curie, Budapest, Hungary), PTH (RIA, Nichols, San Juan, USA) levels were evaluated by applying kits, while serum calcium and phosphate levels were measured by a colorimetric method. Normal ranges of serum calcium and phosphate levels are 2.2-2.6 mmol/l and 0.7-1.6 mmol/l, respectively.

The statistical analyses were performed with a Statgraphics 5.1 program package. A quotient was constituted from the individual basal pre- and postoperative values in order to get normal distribution. For the estimation of pituitary responsiveness the next formula was used:

$$\frac{\text{postoperative peak value}-\text{postoperative basal value}}{\text{preoperative peak value}-\text{preoperative basal value}}$$

To verify the normal or non-normal distribution of the individual parameters, the skewness and kurtosis and their significance were analysed.

In case of normal distribution Student's paired t-test was used. Mean $\pm$ SD are shown. If the distribution was non-normal even after transformation, Wilcoxon signed-rank test was used (for analysing maximal responsiveness).

Pearson's or Spearman's correlation method was used depending on the normality of the parameters.

Results

The parathyroid adenoma of all the patients involved were removed as shown by histological examination.

After surgery the previously high serum calcium levels (3.08 ± 0.27 mmol/l) decreased and the previously low serum phosphate levels (0.65 ± 0.09 mmol/l) elevated (se calcium: 2.08 ± 0.21 mmol/l, se phosphate: 0.95 ± 0.26 mmol/l); the change was highly significant in both cases ($P < 0.001$). The individual serum calcium and phosphate values are shown in Table I. The abnormal PTH levels were also reduced significantly after the operation; before: 430 ± 3.74 µg/l; after: 0.44 ± 0.21 µg/l ($P < 0.01$).

The changes of basal hormone values are shown in Table II, and that of the stimulated values are presented in Table III.

The basal and stimulated postoperative TSH levels showed significant increase. After the removal of the adenoma, stimulated PRL was also higher in patients whose basal PRL levels were previously normal (less than 20 µg/l). There was no difference between basal PRL levels before and after surgery. No significant changes were found in patients with hyperprolactinaemia (data are not shown). Neither the basal FSH and LH secretion in both sexes, nor the stimulated FSH and LH release in men changed after surgery.

Table I

Individual serum calcium and phosphate values

No.	Se Ca^{2+} before operation mmol/l	Se Ca^{2+} after operation mmol/l	Se P^{3-} before operation mmol/l	Se P^{3-} after operation mmol/l
1	3.20	1.90	0.70	0.50
2	3.05	2.30	0.50	0.80
3	3.30	2.15	0.50	0.90
4	2.80	2.40	0.70	1.00
5	3.50	1.60	0.60	0.70
6	2.90	2.30	0.60	0.90
7	3.40	2.00	0.60	0.80
8	3.50	2.00	0.70	1.30
9	2.90	2.00	0.80	1.10
10	2.80	1.90	0.70	1.20
11	3.10	2.00	0.60	1.10
12	2.70	2.05	0.70	1.40
13	2.90	2.10	0.80	0.70

Table II

Changes of basal hormone secretion after surgery

Ratio[a]	n	Mean	95% conf. limit		t	P
TSH	13	2.39	1.37	3.42	2.96	**
nPRL[b]	10	2.23	0.85	3.60	2.02	NS
GH	13	3.05	1.44	4.65	2.77	**
fFSH[c]	4	1.19	0.87	1.51	1.91	NS
mFSH[d]	6	1.13	0.56	1.70	0.60	NS
fLH[c]	4	0.99	0.57	1.41	-0.10	NS
mLH[d]	6	2.01	0.33	4.36	1.12	NS

[a]Ratio = postoperative basal values/preoperative basal values; [b]nPRL = normoprolactinaemic patients; [c]f = postmenopausal female patients; [d]m = male patients; **= $P < 0.02$; NS = non significant (Student's paired t-test)

Table III

Changes of pituitary responsiveness after surgery

Ratio[a]	n	Median	z score	P
TSH	13	1.33	2.06	*
nPRL[b]	10	1.83	2.45	**
fFSH[c]	4	0.50	2.01	*
mFSH[d]	6	0.80	0.21	NS
fLH[c]	4	0.61	1.64	NS
mLH[d]	6	0.79	0.62	NS

[a]Ratio = postoperative peak values—postoperative basal values/preoperative peak values—preoperative basal values; [b]nPRL = normoprolactinemic patients; [c]f = postmenopausal female patients; [d]m = male patients; *= $P < 0.05$; **= $P < 0.02$; NS = non significant (Wilcoxon signed-rank test)

On the other hand, in women after menopause the FSH responsiveness showed a significant decrease ($P < 0.05$), while the reduction of stimulated LH values was not significant. Statistical evaluation was impossible because of the small number of fertile aged women. The basal GH secretion increased significantly after surgery ($P < 0.02$).

There was no apparent correlation between the decrease of calcium and PTH values and the basal and stimulated pituitary hormone secretion.

Discussion

In our study, the biologically and statistically significant decrease in serum calcium and PTH levels and the increase in serum phosphate level could be attributed to the successful removal of the adenoma inducing hyperparathyroidism. The parallel increase in the basal and stimulated secretion of some anterior pituitary hormones underlines the modifying role of extracellular calcium level. In human investigations, TSH response was studied most extensively; acute experimental hyper- and hypocalcaemia and other conditions with chronically altered calcium level change on the TSH response were also observed /7--9, 15/. The results of the present study agree with those of the above examinations: in patients with hypercalcaemia, both basal TSH secretion and its reaction to TRH stimulation decreased. The exact explanation of this phenomenon is not yet known, but there are several experimental data helping in interpretation. It has been revealed that extracellular hypercalcaemia can stabilize secretory granules in pituitary cells /11/ and can stimulate dopamine release from hypothalamic cells, which, on the other hand, may inhibit the release of certain pituitary hormones. Consequently, the administration of metoclopramid could partly suspend the inhibiting effect of hypercalcaemia on TSH release /15/. It seems that the effect of hypercalcaemia involves both indirect and direct inhibition of hormone release. As the referred studies and our examinations demonstrate, the alteration in TSH secretion seems to be independent of negative feedback mechanism, since serum thyroxine and tri-iodo-thyronine levels do not alter.

However, the background of this observation has remained unclear; Gillet et al. /8/ presume that thyroid sensitivity increased by TSH or increased disintegration of the thyroid hormone, even the relative growth of the biologically active form of TSH are all possible in patients with hypercalcaemia.

As to prolactin, the results of human tests are disputed /1, 2, 6, 14, 18, 22/, but most studies suggest an inhibitory effect of exogenic or endogenic hypercalcaemia on basal and TRH stimulated PRL secretion. In the present study we obtained similar results: in patients with normoprolactinaemia the PRL secretion increased significantly after surgery. On the other hand, in patients with hyperprolactinaemia we observed no significant changes following the operation. There is no accepted explanation for this observation yet; it may be supposed that the autonomous process resulting in hyperprolactinaemia is more independent from calcium-regulation.

There are only few data on changes of GH and gonadotrop hormone secretion in human patients with hypercalcaemia. In a report of Veldhuis et al. /22/, TRH + GnRH stimulation test was performed after induction of acute exogenic hypercalcaemia by calcium infusion. These authors found that basal FSH and LH levels did not change in healthy men, yet stimulated secretion was significantly higher than in the control subjects. We found no significant changes in male patients; however, in postmenopausal women higher stimulated gonadotrop hormone secretion was observed before operation (i.e. in hypercalcaemia), than after surgery.

While GH values showed no significant modification in other studies in humans /22/, we observed an increase in basal GH secretion following the decrease of the elevated serum calcium level.

In addition to the change of the extracellular calcium level, the effect of the decreased PTH secretion on the release of certain pituitary hormones could also play a regulatory role. However, the participation of parathromone in the process was not proved either by our results or by other publications /8, 9/, in spite of the fact that an increase in PRL level, induced by PTH infusion, was found in healthy subjects /16/.

We conclude that in humans extracellular calcium level can reversibly modify and regulate the secretion and sensitivity of certain pituitary hormones.

Acknowledgements: This work was supported by a grant of the Hungarian Medical Research Council (T-10/455).

The authors are grateful to András Paksy M.D. for valuable assistance in statistical procedures.

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Acta Medica Hungarica, Vol. 50/1—2, pp. 23—32 (1994)

ATRIAL NATRIURETIC PEPTIDE (ANP) RESPONSIVENESS IN PATIENTS WITH HYPOTHYROIDISM

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(Received: December 23, 1993)

Hypothyroidism is known to be associated with abnormalities of kidney function; recently, low atrial natriuretic peptide (ANP) plasma levels have been reported. Aim of the study was to asses ANP, sodium and water responsiveness to an acute saline load. Twelve patients with established primary hypothyroidism and 9 control subjects were studied. ANP was determined in plasma by RIA with extraction, prior to and after the infusion of saline, 500 ml/h for 4 hours. On a similar albeit liberal sodium diet hypothyroid patients excreted less sodium and water (74 ± 33 (SD) μmol/min and 0.69 ± 0.15 ml/min, respectively) than control subjects (110 ± 52 μmol/min; $P < 0.05$ and 1.06 ± 0.53 ml/min; $P < 0.025$, respectively). However, the infusion of saline resulted in a 3-fold increase of sodium output and more than 2-fold increase in urine flow. The exaggerated responsiveness in sodium excretion in patients with hypothyroidism was associated with significantly decreased pre-infusion ANP plasma levels (16.1 ± 11.1 pg/ml vs. 44.4 ± 14.4 pg/ml; $P < 0.001$) and also with sluggish response to the volume expansion (+ 24% vs. + 48%). A significant correlation was found between serum T4 levels and plasma ANP concentrations in 8 patients ($r = 0.689$; $P < 0.05$). Although hypothyroid patients tend to retain sodium on a liberal salt diet, their kidney is capable of vigorously eliminating excess sodium when challenged with an acute saline load. This exaggerated responsiveness of sodium excretion can be demonstrated in spite of a sluggish response in ANP. Subnormal ANP levels in hypothyroidism are probably the result of thyroid deficiency.

Keywords: Atrial natriuretic peptide, sodium excretion, water excretion, hypothyroidism

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Abbreviations: ANP: atrial natriuretic peptide, RIA: radioimmunoassay, T4: thyroxin, TSH: thyroid stimulating hormone

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Introduction

Hypothyroidism is known to be associated with abnormal sodium and water metabolism. Body sodium was found to be increased /1, 34/ and to diminish upon substitution therapy /26/. Various abnormalities of the renal handling of sodium and water recognized by early clinical studies /15, 28, 35/, and confirmed by subsequent clearance and micropuncture experiments /10, 25/. Recently, it has been shown by several investigators that plasma levels of atrial natriuretic peptide (ANP) are depressed in hypothyroid patients /39, 42/ and rats /20, 38/. Since the release of ANP from the cardiac myocytes may be altered in various chronic sodium-retatining disorders, e.g., renal failure /33, 36/, diabetes mellitus /7/, it seemed worth investigating the effect of an acute saline load on ANP plasma levels and urinary sodium and water excretion in patients with hypothyroidism.

Patients and Methods

Experiments were undertaken in 12 female patients ages 33 to 68 years with established hypothyroidism; all had elevated TSH and low T4 levels. Serum TSH was measured by a sensitive immunoradiometric assay, and T4 by RIA. None of the patients had hypertension as defined by WHO criteria (> 160/95 mmHg, phase V. of the Korotkoff sounds), and detailed clinical and laboratory investigation excluded the possibility of congestive heart failure or any other diseases (renal, hepatic, endocrine) that could have independently affected sodium homeostasis.

The control group consisted of 9 females aged 33 to 59 years, who were free of clinical or laboratory evidence of any circulatory, renal, hepatic or endocrine disorders. Patients and control subjects were hospitalized at the time of the study and received a balanced diet containing daily 150 to 200 mmol of sodium. All gave informed consent to the experiments.

After having collected their 24-hour urine, the patients and subjects remained recumbent for 30 min, at the end of which blood pressure was taken 3 times with sphygmomanometer at 2 min intervals, and the pulse rate was determined. An indwelling cannula was then inserted into an antecubital vein and blood was withdrawn for measurement of ANP, sodium, potassium and creatinine concentrations. After voiding, a 4-hour infusion of physiological saline was started at a rate of 500 ml/hour, at the end of which blood pressure and pulse rate were again measured, and blood sample taken for ANP. The 4-hour urine was collected by spontaneous voiding.

For the determination of ANP, blood was withdrawn into chilled EDTA tubes, centrifuged immediately and the plasma stored at -20 ^{0}C until assaying. Extraction was done using Sep-Pak C 18 cartridges, and radioimmunoassay was undertaken in duplicate, as described previously /30/. Serum and urine electrodes, and creatinine concentration was determined by an autoanalyser. Statistical analysis of the data was performed by using Student's t-test of paired and unpaired samples as appropriate, and by correlation analysis by the method of least squares.

Results

Relevant clinical and biochemical data of the patients and control subjects are shown in Table I. Hypothyroid patients had significantly lower pulse rate and serum sodium level.

Table II demonstrates that patients with hypothyroidism had significantly lower urine flow and sodium excretion compared with the control individuals. During the infusion of 2000 ml saline urinary sodium excretion

Table I

Clinical and laboratory data of patients with hypothyroidism and control subjects (mean ± SD)

	Patients n=12	Control subjects n=9
Age (years)	48.2 (33—68)	44.6 (20—50)
Body weight (kg)	67.0 ± 9.6*	64.1 ± 9.1
Body height (cm)	160.3 ± 5.6	158.7 ± 6.1
Blood pressure (mmHg)	129/82 ± 17/10	125/79 ± 16/8
Pulse rate (beats/min)	67 ± 12**	75 ± 10
Serum sodium (mmol/l)	140.2 ± 3.1*	145.3 ± 3.7
Serum potassium (mmol/l)	4.2 ± 0.9	4.4 ± 0.4
Creatinine clearance (ml/min)	94.41 ± 9.6	98.3 ± 7.1

*$P < 0.05$; **$P < 0.01$ compared with control subjects

Table II

Changes in urine flow (V/min) and sodium excretion ($U_{Na}V$) following the infusion of 2000 ml saline for 4 hours in patients with hypothyroidism and control subjects (mean ± SD)

	Patients n=12	Control Subjects n=9	P
V/min (ml/min)			
Before infusion	0.69 ± 0.15	1.06 ± 0.53	0.001
After infusion	1.62 ± 0.61**	2.04 ± 0.85*	N.S.
$U_{Na}V$ (μmol/min)			
Before infusion	74 ± 33	110 ± 52	0.05
After infusion	228 ± 87**	237 ± 53**	N.S.

*$P < 0.01$; **$P < 0.001$ compared with before infusion values

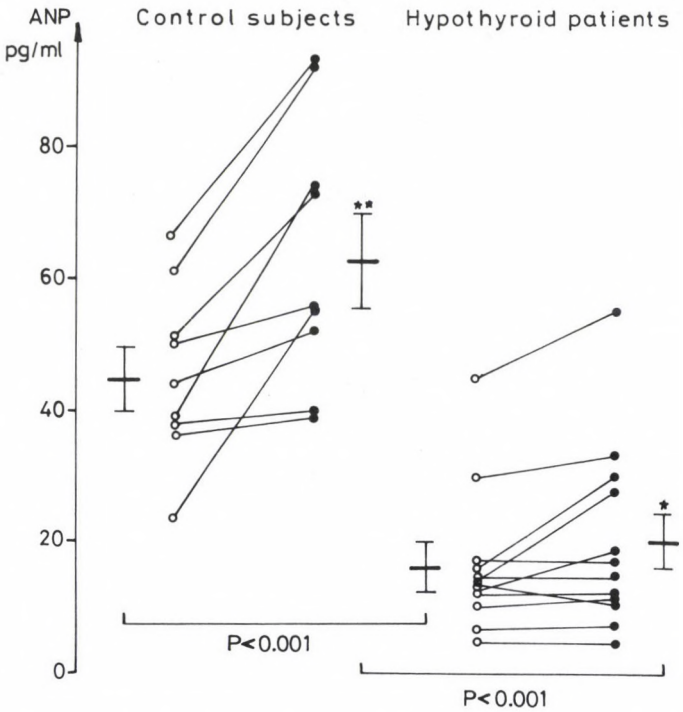

Fig. 1. Atrial natriuretic peptide (ANP) plasma levels before (open circle) and after (solid circle) volume expansion with 2000 ml saline for 4 hours in control subjects and in patients with hypothyroidism. Mean ± SE are indicated; asterisks denote level of significance of changes within a group ($^{**}p < 0.01$; $^{*}p < 0.025$)

was doubled in the control subjects, while a threefold increase was observed in the hypothyroid patients, whose mean sodium output thus approached that of the control subjects. Urine flow was increased by saline infusion to about the same extent in the two groups.

Pre-infusion ANP level in the hypothyroid patients was significantly less than corresponding values of the control subjects (mean ± SD) (16.1 ± 11.0 vs. 44.4 ± 14.4 pg/ml; $p < 0.001$), and it rose significantly in response to volume expansion ($p < 0.025$), although to significantly lower values than those observed in the control subjects (post-infusion means, 20.0 ± 14.3 vs. 62.7 ± 22.0 pg/ml; $p < 0.001$). Figure 1 shows individual responses in both groups.

In 8 patients with hypothyroidism pre-infusion ANP plasma concentration correlated significantly with serum T4 level (Fig. 2), while there was somewhat weaker correlation between serum T4 and post-infusion ANP con-

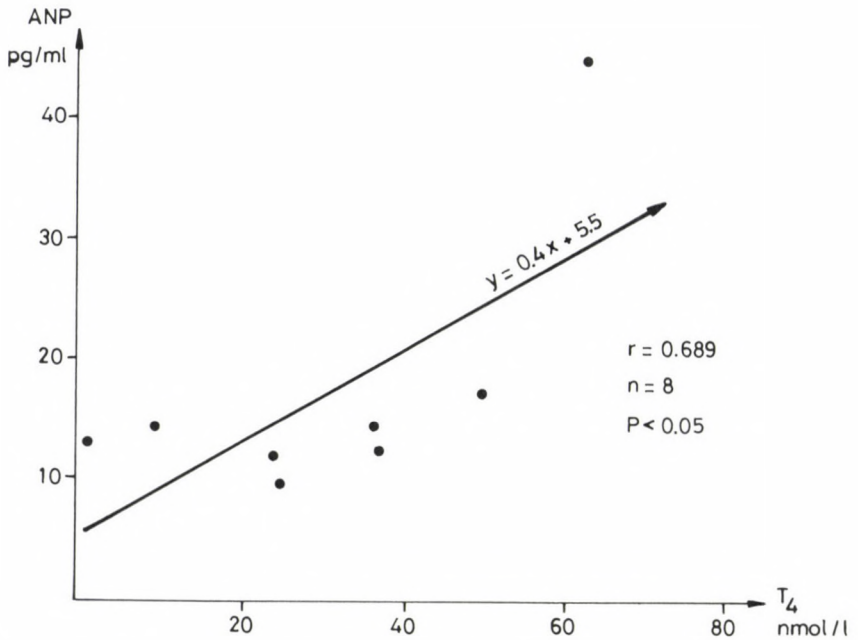

Fig. 2. Correlation between serum T4 level and plasma concentration of atrial natriuretic peptide (ANP) in patients with hypothyroidism

centration (r = 0.600; N.S.) and no apparent relationship between T4 levels and percent changes in the concentration of ANP in response to volume expansion (r = -0.088; N.S.). Plasma ANP level correlated only weakly with the pulse rate (r = 0.360; N.S.). Also there was no correlation between serum sodium concentration and plasma ANP level (r = 0.005; N.S.).

Discussion

The present results confirm earlier observations that hypothyroidism is associated with various abnormalities of kidney function resulting in a tendency to sodium and particularly, to water retention /5, 9, 15, 35/. Under steady-state conditions, our patients with hypothyroidism had significantly lower urinary sodium and water excretion rates than the control subjects, although the two groups were given similar diets. The difference between the patients with hypothyroidism and the control subjects was particularly prominent with respect to the urine flow rate, which is in agree-

ment with the observation that free water formation is impaired in hypothyroidism /10, 25/. Water retention results in hyponatraemia which could also be demonstrated in our patients.

Impaired water excretion in hypothyroidism has been attributed to inappropriately high antidiuretic hormone (ADH) levels /28, 32/, to decreased fluid delivery to the distal diluting segment of the nephron /9/ and to other, less defined abnormalities of kidney function /17/. ADH level was not measured in the present experiment, however, the observation that volume expansion resulted in a more than twofold increase in urine flow in the hypothyroid patients, similar to that observed in the control subjects in response to the same volume load, argues against the possibility that excessively high and not readily suppressible ADH levels /32/ would be responsible for chronic water retention and hyponatraemia in hypothyroidism.

Data concerning the excretion of sodium in hypothyroidism are rather controversial. Michael et al. found that absolute and fractional excretion of sodium was increased in the hypothyroid rats /25/, while Capasso et al. observed decreased excretion in thyroidectomized rats /4/. The patients with hypothyroidism studied by us also excreted significantly less sodium than control subjects. Although both groups were instructed to avoid sodium at the table and they were given similar hospital diets, the possibility cannot be ruled out that there were differences in salt preference.

Several explanations have been provided for the sodium retention occurring in hypothyroidism. Thus Di Scala and Kinney /10/, and Gibson et al. /13/ suggested that enhanced proximal tubular reabsorption of sodium caused by diminished blood volume might be responsible for observed abnormality of sodium and water excretion. Capasso et al. /4/, on the other hand, thought that it was the increased intrarenal angiotensin II level which could have acted as a sodium retaining factor. Whatever the underlying mechanism, it is clear that, despite the tendency for sodium retention under steady-state conditions, hypothyroid rats respond with remarkable natriuresis to the infusion of saline /18/, similarly to the patients observed by us. When challenged with an acute saline load, our patients exhibited a 3-fold increase in sodium excretion. Thus, taking together these data with the results of water excretion, it may be concluded that acute volume regulatory responses are well maintained in hypothyroidism; moreover, the pronounced natriuretic and diuretic responses occur in face of subnormal resting ANP levels and a slugghish responsiveness of ANP release in these patients.

Low plasma ANP concentration in patients with hypothyroidism has been reported by several /21, 39, 42/, although not all investigators /22, 41/. Of the possible mechanism underlying this abnormality the frequent occurrence of pericardial effusion in hypothyroid patients has been suggested to be responsible for the low ANP levels found some of the patients /41/; in fact, increased atrial pressure due to pericardial effusion in hypothyroid patients /16/ may impede atrial stretch which regarded to be the main stimulus for ANP release /11/. However, Weissel et al. /37/ found positive ANP level in hypothyroid patients.

A further possibility related to the cardiac abnormalities of hypothyroidism that could explain low ANP levels in this disease is bradycardia. Plasma ANP level depends on heart rate during ventriculoatrial pacing /12/, and Kohno et al. /21/ found significant correlation between ANP concentration and heart rate in patients with hyperthyroidism. In the present study there was only weak, statistically not significant correlation between pulse rate and resting ANP levels, thus it seems unlikely that bradycardia would be the primary cause of low ANP concentration in hypothyroidism.

Since serum sodium concentration is low in hypothyroidism /1, 28/ and this could be demonstrated also in the present study, one might suggest that low ANP level be the result of decreased serum concentration of sodium in these patients. The sodium ion has been incriminated as the principle stimulus for ANP release /3/, however, in a recent study elevation of plasma sodium concentration without volume expansion failed to affect ANP release /27/. In the hypothyroid patients studied by us there was no correlation between serum sodium concentration and plasma ANP level.

Finally, it has been suggested that diminished thyroid hormone production be responsible for the suppression of ANP in hypothyroidism /21, 42/. This view is supported by the observation of Ladenson et al. /23/ showing that the ANP messenger RNA in the myocardium is reduced in hypothyroid rats and can be stimulated by the administration of thyroxine. Treatment of hypothyroid patients with thyroxine resulted in significant increases in plasma ANP level /21/, even in patients in whom initial ANP concentration was not suppressed /41/. The finding of a close correlation between serum T4 level and plasma ANP concentration in the study of Kohno et al. /21/ and in the present experiments lends strong support to the view that thyroid hormones are important modulators of ANP synthesis.

The most important finding in the present study is the demonstration of not only low plasma ANP levels but also of a diminished responsiveness of

ANP in patients with hypothyroidism. It is well established that saline infusion enhances ANP release /21, 24, 40/; the rate of release, however, seems to depend on the actual sodium status, usually being high in conditions associated with sodium retention /7, 33, 36/. We also succeeded in demonstrating that ANP responsiveness depends on exchangeable sodium /8/. Since body sodium has been shown to be increased in patients with hypothyroidism /1,34/, one would expect an exaggerated ANP response to an acute colume expansion; however, this was not the case, the present increase of plasma ANP level in our patients following the infusion of saline attained only 24% compared with 48% observed in the control individuals. We believe that deficient thyroid hormone production is responsible also for this abnormality of ANP release in hypothyroidism.

Although enhanced secretion of ANP is regarded by most investigators as being instrumental in the renal elimination of an acute saline load /2, 19, 24/, it seems that renal mechanism independent of ANP may be equal or even greater importance /29, 31/. Especially in states of chronic sodium retention, volume expansion may fail to enhance ANP release, despite an increase in atrial diameter /6/. In hypothyroid dogs, Zimmerman et al./43/ observed marked increases in sodium excretion despite low ANP levels. In the present study hypothyroid patients responded with an even more vigorous enhancement of renal sodium excretion than did the control subjects to the same standardized volume load. The fact that this occurred in face of low resting and stimulated ANP levels and sluggish increase following the saline infusion indicates that, in hypothyroidism, mechanism other than ANP release are mainly responsible for the elimination of an acute sodium load.

Acknowledgements: We are grateful to Ms. Edit Sándor, Ms. Ágnes Gábri, Ms. Ilona Takács and Ms. Ilona Jókai for their skillfull technical assistance.

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Acta Medica Hungarica, Vol. 50/1—2, pp. 33—42 (1994)

CARDIOLOGY

CHARACTERISTICS OF LONG QT WITH PERMANENT BRADYCARDIA

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(Received: December 1, 1993)

The study was aimed to investigate the electrophysiological properties of long QT syndrome associated with permanent bradycardia. The investigations were performed in 26 patients suffering from long QT duration (QT_C — frequency adapted QT —: 484 ± 34 ms) with permanent, marked bradycardia (heart rate: 42 ± 7 min^{-1}). Adams Stokes syncopal attack appeared in 12 patients, while in 14 cases ventricular tachycardiac attack with syncope could be observed (study group). As control served the data of 30 patients suffering from long lasting marked bradycardia (heart rate: 44 ± 7 min^{-1}) with normal QT (QT_C: 420 ± 28 ms). Each patient was candidate for pacemaker implantation. The following questions were studied: 1. The effect of heart rate on QT duration. The experiments were performed by electrical ventricular stimulation. 2. The effect of sympathetic and parasympathetic — pharmacologic — blockade on QT time. The study was performed under electrical ventricular stimulation by administration of propranolol and atropine. 3. The dispersion of QT time was studied by using electrical heart stimulation and 12 lead ECG recording.

Electrophysiological investigations were performed in 14 patients with long QT and permanent bradycardia. On augmentation of the cycle length (bradycardia) the increase in the QT duration was more — out of all proportion — expressed in long QT. On pharmacologic sympathetic blockade in long QT syndrome the QT duration significantly diminished. The QT dispersion was more expressed in patients with prolonged QT interval and on bradycardia the QT dispersion further increased significantly. The irritability of the ventricle was markedly augmented in patients with long QT and bradycardia. Appearance of polymorphous ventricular tachycardia could frequently be observed and could be regularly induced by early ventricular extrastimuli and bradycardia. The permanent bradycardia seems to modify the electrophysiological characteristics of long QT and increase its arrhythmogenic property. According to our study the late outcome of long QT with bradycardia is favourable by application of pacemaker implantation (using higher pacing rate) and beta-blocker administration.

Keywords: Long QT syndrome, dispersion of the QT interval, bradycardia, heart rate-QT relationship, ventricular irritability, sympathetic blockade

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Abbreviations: LQTS: long QT syndrome, CL: cycle length, QT: QT interval, QT_C: frequency adapted QT interval, EP: electrophysiological study, PMI: pacemaker implantation, VERP: ventricular effective refractory period, AERP: atrial effective refractory period

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Introduction

The prolongation of the QT interval is an important predictor of serious ventricular tachycardias. The long QT syndrome (LQTS) represents a special entity of arrhythmogenic property. Despite many clinical and experimental studies the pathomechanism of the ventricular irritability in LQTS could not yet correctly be revealed. The association of LQTS with permanent bradycardia is not a rare phenomenon /11, 13, 20, 23, 25/.

Scanty clinical observations and experimental data suggest that on bradycardia the arrhythmic propensity of LQTS can be modified /9, 25/. This study was designed to investigate the effect of bradycardia on the arrhythmogenic character and electrophysiological characteristics of LQTS. To study the long-term therapeutical effect of pacemaker implantation with administration of beta-blockers in LQTS with bradycardia was the other objective of the study.

Patients and Methods

Twenty-six patients suffering from LQTS and permanent — marked — bradycardia could be enrolled in the study (study population). Exclusion criteria were: 1. Serious accompanying heart disease (coronary artery disease, cardiomyopathy, congenital or acquired valvular heart disease). 2. Progressive cerebrovascular disease, renal or hepatic insufficiency. All patients were referred to our clinic for pacemaker implantation i.e. to determine the proper therapy. The most important clinical data of the study group were summarized in Table I.

To compare the experimental and clinical results the adequate data of 30 patients suffering from marked long-lasting bradycardia (control group) served as control, each patient was candidate for pacemaker implantation. The exclusion criteria in selection of patients were the same as those in the study group. The clinical characteristics of the control group were depicted in Table II.

The patients of the two groups were quite well-matched for age, sex distribution and heart rate. It was remarkable that in the study group the appearance of major ventricular arrhythmias was frequent, demonstrating the propensity for ventricular irritability in LQTS with bradycardia. There was — as could be expected — great difference in the QT between the two groups.

The QT interval was measured from the onset of QRS to the end of T wave at ECG paper recording speed 50 $mm \cdot s^{-1}$ and the average of three consecutive beats was taken. The end of T wave was defined as return to the TP base line. When U wave interrupted the T wave before return to base line, the QT interval was measured to the nadir of the curve between T and U waves /14/.

The frequency adapted QT (QT_C) was determined by using the Bazett's formula /2/. Long QT duration was recognized when QT_C was markedly prolonged: QT_C more than 460 ms. The effect of heart rate on QT interval was studied in 24 patients; in 14 cases from the study group and in 10 patients from the control group. The study was performed before the pacemaker implantation to select the proper pacemaker frequency rate. The QT time was measured by using different pacing — driving — cycle length (600—1100 ms).

Table I

Clinical characteristics of patients suffering from permanent bradycardia associated with long QT duration

Patients (N):	26
Age (years):	28 ± 6 (range 12—42)
Sex (male/female):	16/11
Heart rate (beats. min^{-1}):	42 ± 6.8 (36—59)
QT_C (ms):	484 ± 34 (470—510)
QT (ms):	630 ± 48 (560—690)
Accompanying diseases	
Hypertension:	1
Diabetes mellitus:	1
Genesis of bradycardia	
Sick sinus syndrome:	1
2nd-3rd degree AV block:	18
Sick sinus syndroma with AV block:	7
Indication for pacemaker implantation	
Adams Stokes syncopal attack:	12
Ventricular tachycardiac attack with syncope:	14
Type of LQTS	
Congenital long QT:	2
Acquired — sporadic — QT:	24

For investigation the effect of sympathetic-parasympathetic interactions on the QT pharmacologic sympathetic and parasympathetic blockade was performed in 26 patients; in 16 from the study group and 10 from the control group. To avoid the disturbing effect of the sympathetic and parasympathetic blockade on the heart cycle length, the investigation was performed under ventricular electrical stimulation using constant — 800 ms — pacing-driving-cycle. For parasympathetic blockade atropine was administered: 0.04 $mg \cdot kg^{-1}$ intravenously. For sympahetic blockade propranolol was applied: 0.2 $mg \cdot kg^{-1}$ in intravenous injection.

The QT dispersion was determined in 16 patients during electrical ventricular heart stimulation by using different pacing-driving-cycle length (in 8 patients with LQTS and in 8 ones from the control group). The QT dispersion was defined as the difference between the maximum and minimum QT intervals measured on the standard 12 lead ECG. Normal value: 48 ± 20 ms /6, 9, 15/.

Electrophysiologic study (EP) was performed in 14 patients suffering from LQTS and long-lasting bradycardia with appearance of polymorphous ventricular tachycardiac attack. The EP consisted of programmed electrical stimulation of the heart and recording endocavital ECG tracings (intracardiac electrograms from the His bundle region, high and low right atrium and different area of the right ventricle). For electrical stimulation a programmable digital stimulator (Medtronic 5325) was used. To induce ventricular tachycardia single and double ventricular extrastimuli were applied. For statistical analyses of the data the Student's t-test for unpaired and paired data was applied.

Table II

Clinical characteristics of patients suffering from permanent bradycardia

Patients (N):	30
Age (years):	35 ± 5 (range: 13—55)
Sex (male/female):	18/12
Heart rate (beats. min^{-1}):	44 ± 7 (36—59)
QT_C (ms):	420 ± 28 (380—440)
QT (ms):	484 ± 30 (450—520)
Accompanying diseases	
Hypertension:	2
Emphysema:	1
Diabetes mellitus:	1
Genesis of bradycardia	
Sick sinus syndrome:	3
2nd-3rd degree AV block:	16
Sick sinus syndrome with AV block:	11
Indication for pacemaker implantation	
Adams Stokes syncopal attack:	20
Permanent bradycardia:	9
Brady-tachycardia:	1

Results

The QT interval was regularly longer in LQTS than in the control group at different pacing rates. On bradycardia (long driving cycle), although the QT increased in both groups, however, the QT prolongation on bradycardia was significantly more pronounced in LQTS. In tachycardia the difference in QT between the two groups proved statistically non significant. On bradycardia the difference became definitely marked and statistically highly significant (Fig. 1).

On a pharmacologic parasympathetic blockade — atropine administration — there was no significant change in the QT using constant pacing rate. At propranolol administration — sympathetic blockade — in normal cases (control group) the QT did not change significantly — at constant pacing rate — while in LQTS significantly shortened (Fig. 2).

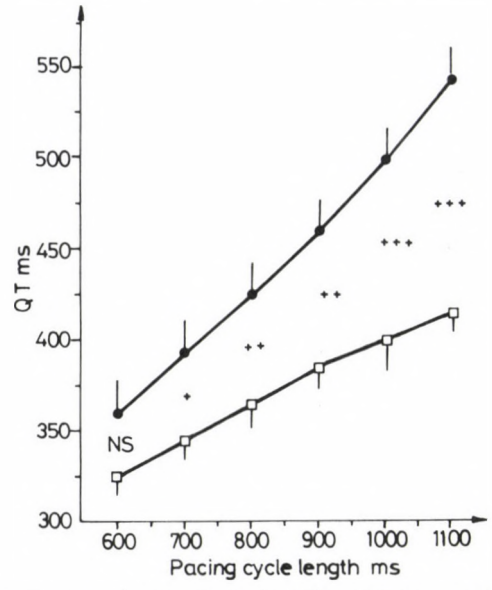

Fig. 1. The effect of heart rate (pacing cycle length) on QT in normal patients and in LQTS. □: patients with normal QT (mean values and SD); ●: patients with LQTS (mean values and SD). Mean values were compared with unpaired t-test. NS: $p > 0.05$; +: $p < 0.05$; ++: $p < 0.01$; +++: $p < 0.001$

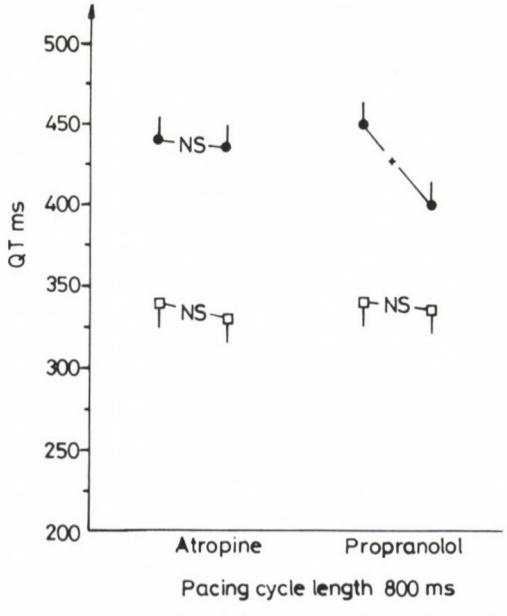

Fig. 2. The result of parasympathetic (atropine) and sympathetic (propranolol) pharmacological blockade on QT in normal patients and in LQTS. □: mean values and SD of patients with normal QT; ●: mean values and SD of patients with LQTS. The initial — basal — values were compared — by using paired t-test — with those after atropine or propranolol administration

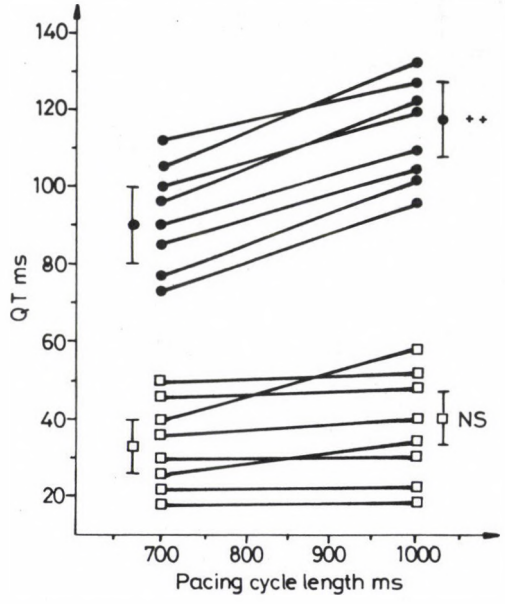

Fig. 3. The dispersion of QT in normal patients and in LQTS: the effect of the increase in pacing cycle length on QT dispersion. □: mean values and SD of patients with normal QT; ●: mean values and SD of patients with LQTS. Paired data t-test was applied to compare the values in 700 ms cycle length with those in 1000 ms cycle length

The results of this investigation suggest that in LQTS sympathetic overactivity can be supposed. The dispersion of the QT in LQTS was increased already under normal heart rate (normal pacing rate). The difference between the minimum and maximum QT time was 40 ms in average in normal cases and it was 90 ms in LQTS. It was also interesting, that on bradycardia the QT dispersion markedly further augmented in LQTS, while in normal cases did not change significantly (Fig. 3).

In LQTS with bradycardia we could frequently observe the appearance of ventricular irritability. In 14 cases of 26 patients recurrent polymorphous ventricular tachycardiac attacks could be detected. The results of the EP furnished further data about the arrhythmic propensity for the heart in LQTS with bradycardia. Sustained (mostly of torsade de pointes type) ventricular tachycardia could be induced by early single or double extrastimuli in 14 patients from 16 cases. It is also worth for mentioning that the QT related ventricular effective refractory period (VERP) was very short; VERP/QT: 0.52 ± 0.07 (normal value according to our measurements in patients with normal QT is: 0.64 ± 0.08). There was not any significant

difference from the normal values concerning the other electrophysiological parameters (VERP, AERP, AV conduction time, AV node Wenckebach points). As treatment of LQTS with bradycardia, each patient was implanted with pacemaker using comparatively high pacing rate: 75-85 pro minute and received beta blocker, too. Two patients received dual chamber pacemaker and two other ones were implanted with rate responsive pacemaker using ventricular pacing mode.

The late outcome was very favourable (follow-up: 10-60 months). No syncopal attack or appearance of ventricular tachycardia could be revealed after the pacemaker implantation. Only in two patients could occasionally ventricular couplets be detected.

Discussion

The LQTS represents a well-recognized, but poorly understood syndrome. The LQTS has two types: 1. Congenital, familiar LQTS. 2. Acquired -- sporadic — LQTS. The familiar LQTS can be divided in two subgroups: LQTS with /13/ and without /21, 27/ deaf-mutism. The characteristic recurrent ventricular tachyarrhythmias accompaniyng the LQTS attracted the interest of many clinicians and physicians. The different clinical and experimental studies furnished quite divergent data about the pathogenesis and mechanisms of ventricular tachycardias in LQTS, but the arrhythmogenic property of QT prolongation received general acceptance. Among the numerous theories to explain the arrhythmogenic propensity for the heart (ventricle) of LQTS two seem to be essential: 1. Some experimental data and clinical observations suggest that a sympathetic imbalance, unilateral change in the sympathetic tone of the heart (lower than normal right sympathetic activity) can frequently be revealed in LQTS /16, 22, 28/. 2. The results of other studies seem to prove that an exaggerated dispersion of ventricular refractoriness a non-uniform and delayed recovery of repolarization represents one of the most important reason for ventricular irritability in LQTS /4, 9, 18, 23/. The results of this study indicate that both of the two mechanisms can be involved in the genesis of ventricular polymorphous tachycardia in LQTS.

There are many studies dealing with the effect of parasympathetic and sympathetic blockade on QT. The majority of investigators agree that on parasympathetic blockade the QT has not changed remarkable /1, 5, 24/. Our present data prove that on atropine administration the QT will not change

neither in LQTS nor in normal cases if the heart rate remains constant (using constant pacing rate). Concerning the effect of sympathetic blockade on QT divergent data exist. In some studies a little shortening of QT could be detected on beta-receptor administration /8, 10, 19/. Other investigators found a small prolongation of QT on beta-receptor blockade /1, 26/. No comprehensive study could be found dealing with the effect of sympathetic blockade on QT at constant heart rate in normal patients and in LQTS. According to our investigation the QT will significantly shorten on sympathetic blockade in LQTS. This finding supports the assumption of a sympathetic overactivity in LQTS.

Inhomogeneous ventricular repolarization is postulated as one of the reason of QT prolongation in LQTS. The widened dispersion of QT in LQTS can be reconcilated with this theory. We also could detect markedly greater dispersion of QT in LQTS, confirming the data of Linker et al. /15/. It should be emphasized that the dispersion of QT significantly, very remarkable augments on bradycardia in LQTS.

According to our study the bradycardia will modify the characteristics of LQTS and will increase the arrhythmic propensity for the ventricle. On bradycardia in LQTS an exaggerated QT prolongation could be revealed in our study. The dispersion of ventricular repolarization also markedly increased on bradycardia. The appearance of polymorphous ventricular tachycardia was very frequent in LQTS associated with permanent bradycardia. We also could regularly induce ventricular tachycardiac attack by ventricular extrastimuli in LQTS with bradycardia. The advent of pacemaker therapy and the administration of beta-blockers significantly improved the prognosis of LQTS. The beta-blocker therapy was comparatively early applied in the treatment of LQTS. The results were quite divergent; in some cases the therapeutical result was good, while in other ones the treatment proved to be ineffective. A prerequisite of a successful treatment of LQTS is to prevent the development of bradycardia. On beta-blocker administration the heart rate slows down. The combination of beta-receptor blocking agents with pacemaker implantation resulted much better therapeutical effect /3, 7, 11, 12, 20, 25/. According to our experiences the late outcome of LQTS with bradycardia is very favourable by using pacemaker implantation with administration of beta-receptor blocker. It is advisable to determine the proper pacing rate before the pacemaker implantation. Generally quite high pacing rate (75-85) is necessary to shorten significantly the QT duration. Because of the very expressed ventricular irritability in LQTS associated with bradycardia the therapy should early be started.

Conclusions, Clinical implication

The results of this study with data of clinical observations allow us to make some conclusions concerning the characteristics of LQTS.

1. The association of permanent bradycardia with LQTS represents a subset group of LQTS.

2. The bradycardia markedly increases the ventricular irritability in LQTS.

3. The dispersion of ventricular repolarization further increases on bradycardia in LQTS.

4. The augmentation of the QT on bradycardia is more — pathologically -- expressed in LQTS.

5. The sympathetic blockade results a significant shortening of QT duration in LQTS.

6. Inhomogeneous ventricular refractoriness and sympathetic overactivity have a part in the arrhythmogenic property of the heart in LQTS.

7. The late outcome of LQTS with permanent bradycardia is favourable by using pacemaker implantation (with higher pacing rate) and beta-receptor administration.

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Acta Medica Hungarica, Vol. 50/1—2, pp. 43—54 (1994)

ANALYSIS OF RE-ENTRY MECHANISM IN A PATIENT WITH CONCEALED WOLFF—PARKINSON—WHITE SYNDROME

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(Received: February 15, 1994)

This report deals with a patient with concealed Wolff—Parkinson—White syndrome in whom both narrow and wide QRS tachycardias were observed. The simultaneous occurrence of various QRS morphology during supraventricular tachycardia results in a challenging diagnosic ECG problem. The cycle length during tachycardia with left bundle branch block was longer than the cycle length during narrow QRS supraventricular tachycardia and with functional right bundle-branch block. Electrophysiologic studies revealed an increased V-A conduction time during tachycardia with left bundle branch block. These studies suggested the presence of a concealed left-sided anomalous pathway. Differentiation between intra- and extranodal re-entry and therapeutic modalities are also discussed.

Keywords: Concealed anomalous pathway, A-V reciprocal tachycardia, retrograde conduction time, aberrant intraventricular conduction

Introduction

Atrioventricular re-entry tachycardias can occur within the AV node or using the normal pathway and an extranodal pathway /8/. In the absence of delta wave in sinus rhythm paroxysmal supraventricular tachycardia (SVT) mostly due to re-entry within the AV node. However there are cases in which anomalous pathway (bundle of Kent) capable of only retrograde conduction and therefore concealed on the surface electrocardiogram, id est delta wave is not present /3, 5, 7, 12--17, 20/. Increase in cycle length of SVT during development of functional bundle branch block could be helpful for making

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Abbreviations: SVT = supraventricular tachycardia, EPS = electrophysiologic study, W.-P.-W. syndrome = Wolf--Parkinson—White syndrome, HRA = high right atrial electrogram, HBE = His bundle electrogram, SNRT = sinus node recovery time, PES = premature extrastimulus

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the diagnosis of concealed extranodal anomalous pathway /2/. Differentiation between AV-nodal re-entry and re-entry via an accessory pathway is based on electrocardiographic, electrophysiologic and clinical observation and has both diagnostic and therapeutic implications /12/. We report herein the findings of a patient whose ECG during sinus rhythm showed no evidence of preexcitation and during tachycardia the wide QRS configuration suggested the existence of ventricular tachycardia. Electrophysiologic study (EPS) was helpful for establishing the diagnosis of concealed Wolff—Parkinson—White (W.-P.-W.) syndrome.

Case report

The patient was a 31-year old male without any evidence of organic heart disease. He had suffered serious recurrent tachycardia for 6 weeks. ECG recording between the attacks have shown sinus rhythm with normal P-Q interval (0.16 s) and a QRS duration of 0.08 s, without delta waves (Fig. 1). Before hospital admission episodes of SVT become more frequent and lasting for several hours. During tachycardia either narrow QRS or right (RBBB) or left bundle branch block (LBBB) configuration of the QRS complex could be observed. Because of differential diagnostic difficulties and failure of drug therapy (propranolol, Digoxin, verapamil) the patient was referred to EPS.

Method

EPS was performed after informed consent was obtained. Cardioactive drugs were discontinued 48 hours prior the study. Multipolar electrode catheters were inserted percutaneously through femoral veins and advanced under fluoroscopic control to positions in the right atrium and AV-junction to record His bundle electrogram (HBE) /19/. Both of atrial and ventricular electrical stimulation were performed. Anterograde and retrograde conduction intervals were recorded during sinus rhythm and during induced SVT. Surface ECG leads (I, II, III), high right atrial electrograms (HRA) and HBE were recorded simultaneously with a paper speed of 50 and 100 mm/s. The atrial stimuli consisted of rectangular impulses of 2.0 ms duration and at twice diastolic threshold. For cardiac stimulation Biotronic UHS-20 electrostimulator was used.

Results

During sinus rhythm HBE revealed normal conduction intervals proximally and distally to the His bundle (A-H interval: 70 ms and H-V interval: 50 ms, respectively). Atrial pacing from different sides failed to demonstrate delta wave due to preexcitation. Sinus node recovery time (SNRT) and its corrected value were within normal range; 1140 ms and 320 ms, re-

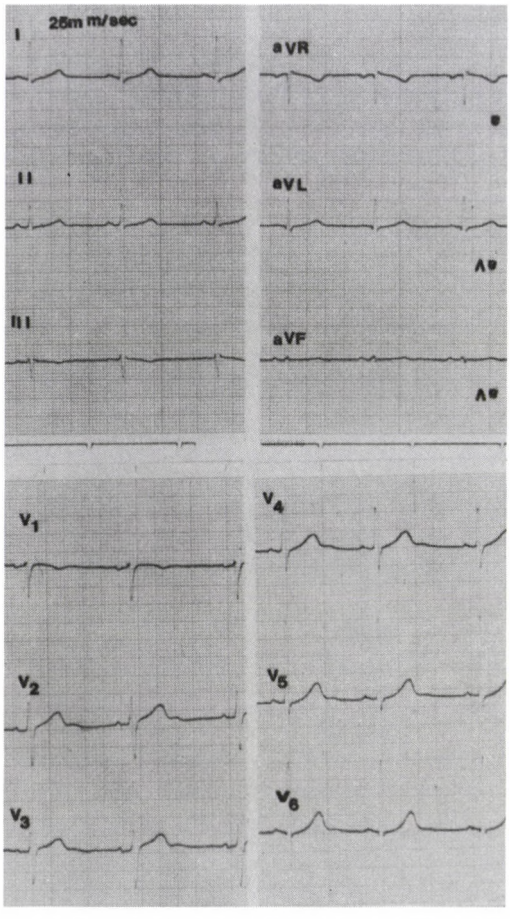

Fig. 1. V. GY., 31 years old male. Twelve leads electrocardiogram (ECG). Sinus rhythm and normal (0.16 s) P-Q interval. QRS also is normal, there is no evidence of preexcitation. Paper speed is 25 mm/s

spectively. During incremental high atrial pacing A-H interval increased from 70 ms to 200 ms, while H-V interval and QRS width remained unchanged. During incremental ventricular pacing (90-150/min) ventriculoatrial retrograde conduction time was fixed (V-A interval: 190 ms).

During atrial extrastimulus testing the following electrophysiologic responses were observed: at sinus rhythm an early atrial extrastimulus (PES) with a coupling interval between 600-440 ms conducted with a prolongation of A-H interval and with narrow QRS complex. A PES with a coupling interval of 420 ms resulted in right bundle branch (RBBB) configuration but without an

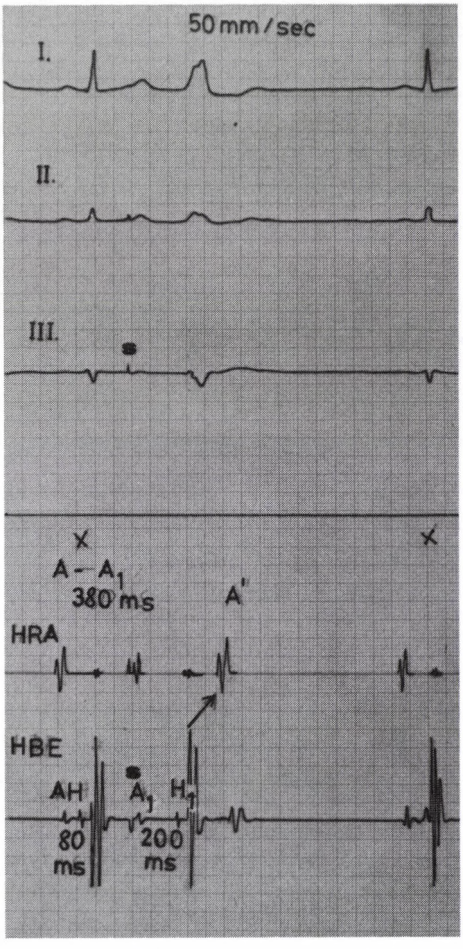

Fig. 2. During sinus rhythm a PES with a coupling interval of 380 ms resulted LBBB. QRS was followed by a trial echo beat (Á). HRA = high right atrium, HBE = His bundle electrogram. Paper speed is 50 mm/s

atrial echo beat. A PES with a coupling interval of 380 ms conducted with 200 ms of A-H interval and resulted in left bundle branch block (LBBB) configuration. Concomitantly atrial echo beat (Á) was demonstrated (Fig. 2). During sinus rhythm a PES with a coupling interval of 340 ms elicited sustained SVT. During SVT the R-R cycle length was 300 ms with a RBBB configuration (Fig. 3). Paroxysmal loss of functional RBBB during tachycardia resulted not any change in R-R interval and retrograde V-A conduction time (Fig. 4). Several episodes of functional LBBB were observed during induced paroxismal SVT. The cycle length and V-A conduction time during tachycardia

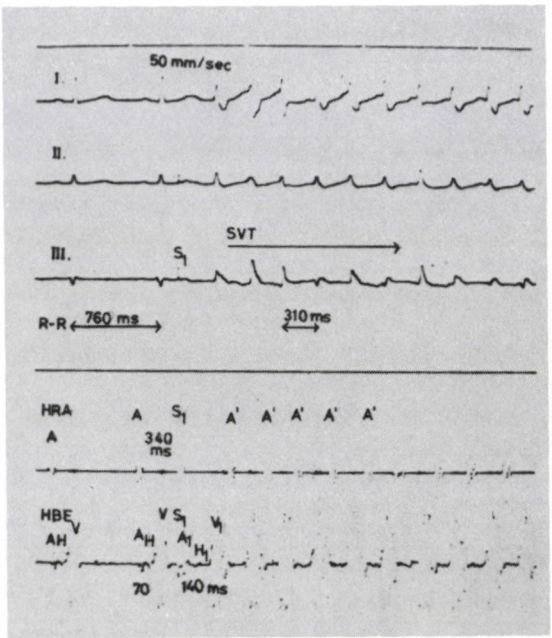

Fig. 3. PES with a coupling interval of 340 ms initiates SVT with functional RBBB. ECG leads I, II, III, HRA and HBE. Paper speed is 50 mm/s

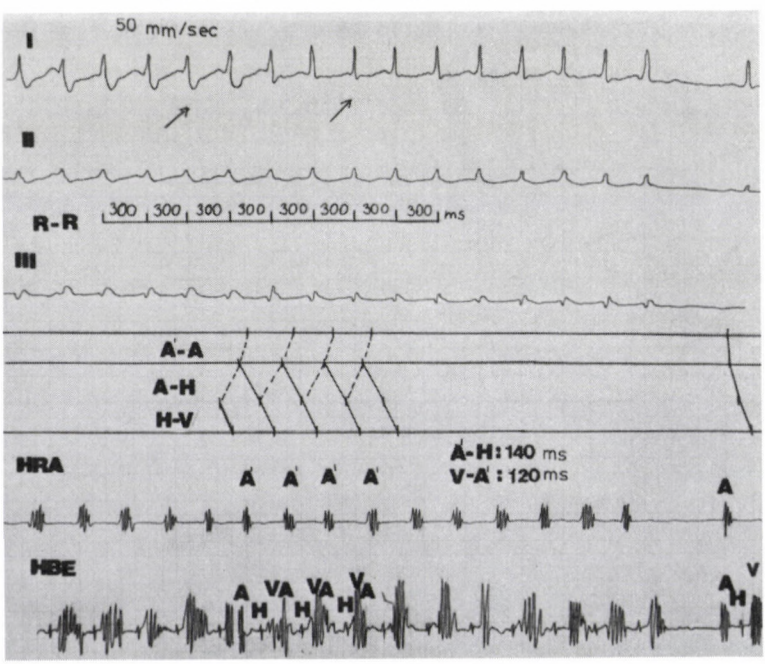

Fig. 4. Sudden loss of RBBB configuration during SVT. Note that cycle length of tachycardia and retrograde conduction time (VA) do not change after morphologic alteration of QRS configuration. ECG leads I, II, III, HRA and HBE. Paper speed is 50 mm/s

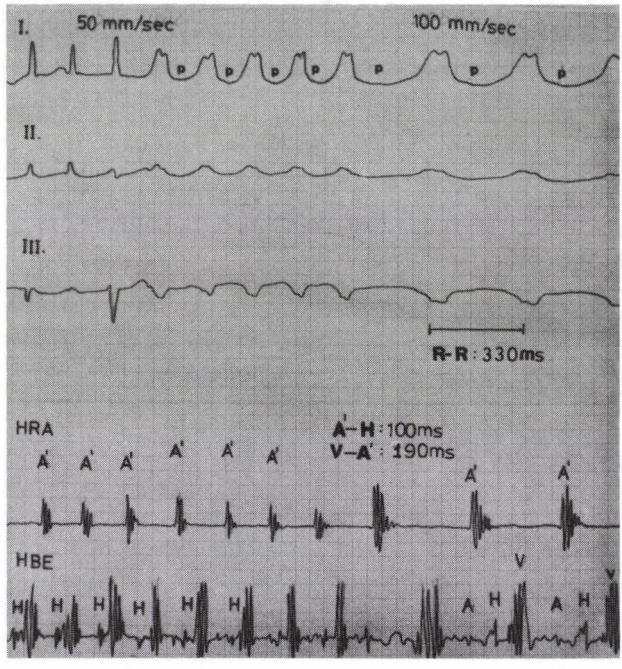

Fig. 5. Occurrence of LBB during SVT suggesting left free wall accessory pathway. Note that cycle length of SVT and VA conduction time increased from 300 ms to 330 ms and from 120 ms to 190 ms, respectively. ECG leads I, II, III, HRA and HBE. Paper speed is 50 mm/s, than 100 mm/s

with LBBB were 330 ms and 190 ms, respectively (Fig. 5). The SVT could be repeatedly terminated with PES, but spontaneous termination of the tachycardias could also demonstrated (Fig. 6). After administration of quinidine, verapamil therapy only non-sustained form of SVT could be induced (Fig. 7). The sustained tachycardia has subsequently been effectively prevented with the above-mentioned medication.

Discussion

Occurrence of SVT with functional BBB results in a challenging diagnostic problem. Re-entry tachycardias are involving the AV node alone or associated with an accessory bypass conduction in the appearance of preexcitation (W.-P.-W. syndrome) /1, 2, 4, 5, 12, 13, 17, 22/. If delta wave is present in the ECG tracing, recorded during sinus rhythm, of a patient with SVT, the tachycardia usually related to the participation of an anomalous

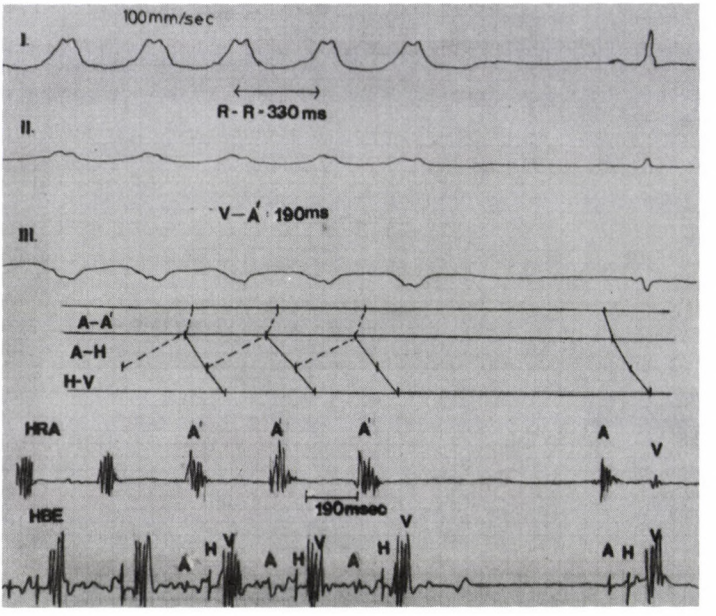

Fig. 6. Spontaneous termination of SVT with functional LBBB. ECG leads I, II, III, HRA and HBE. Paper speed is 100 mm/s

pathway. In the absence of delta wave in sinus rhythm SVT is based regularly on re-entry within the AV-node. However in many patients the SVT is related to an accessory pathway which conducts only in retrograde (VA) direction, therefore concealed on surface electrocardiogram. EPS is helpful in differentiation between intra- and extranodal re-entry tachycardias and has as diagnostic as therapeutic value.

In our presented case the presence of concealed anomalous pathway was suggested by the following:

1. On 12 leads surface electrocardiogram there was not any evidence of preexcitation (normal P-R interval, or delta wave),

2. atrial pacing from multiple sides failed to elicit delta wave due to preexcitation,

3. at increasing rates of ventricular stimulation the retrograde conduction time (VA interval) remained constant (190 ms), /11/,

4. during SVT with LBBB pattern increase in R-R interval and VA-interval could be observed from 300 ms to 330 ms and from 120 ms to 190 ms, respectively.

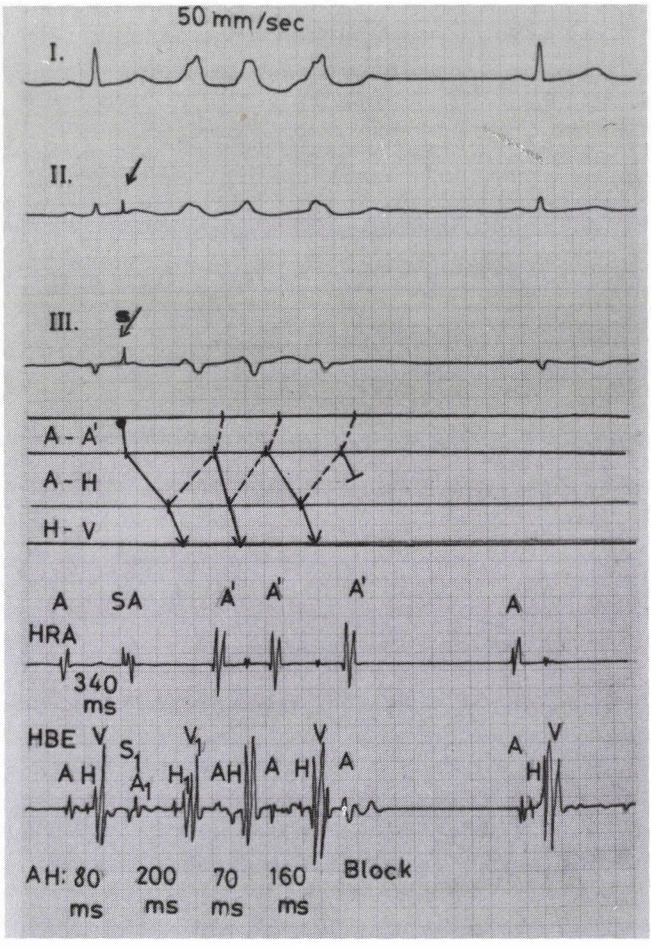

Fig. 7. After administration of quinidine, verapamil therapy only non-sustained from of SVT could be initiated with PES. ECG leads I, II, III, HRA and HBE. Paper speed is 50 mm/s

All of the above electrophysiologic features could be detected in our case suggesting the presence of a concealed accessory pathway in the left free wall of the heart (Fig. 8). The following electrocardiographic and clinical features seem to exclude the diagnosis of AV-junctional re-entry tachycardia with aberrant ventricular conduction:

1. During PES we could not demonstrate dual pathway AV-nodal function curves, which are very characteristic for the mechanism of re-entry in the AV-node.

2. During tachycardia with AV-node re-entry the retrograde P(Á) mostly located in R(V). In our case P waves occurred after QRS and were negative in

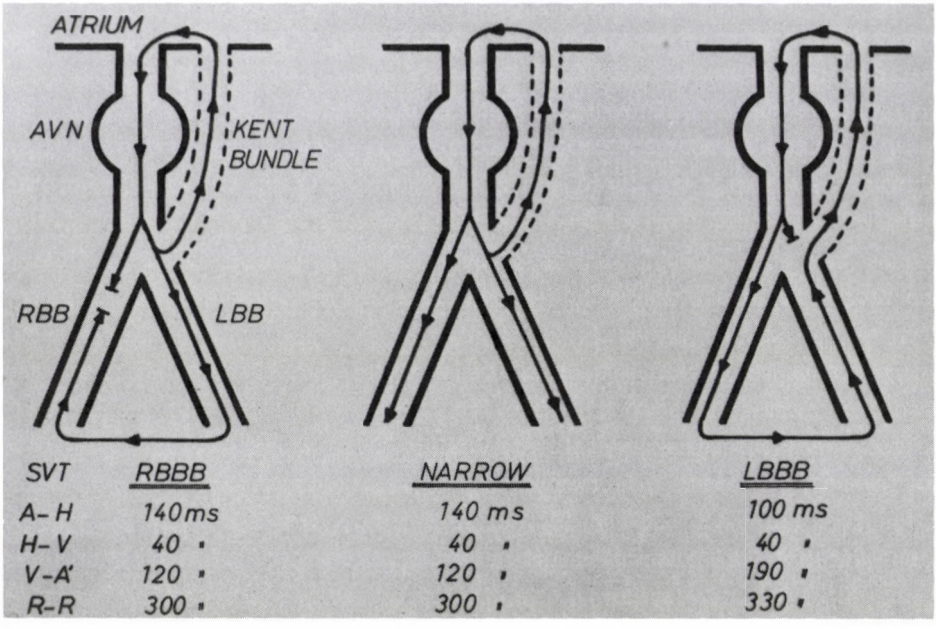

Fig. 8. Schematic representation of mechanism of re-entry tachycardia with narrow QRS, with RBBB and LBBB configuration. Measurement of anterograde and retrograde conduction intervals are presented. Note that during SVT with LBBB configuration the expansion of re-entry cycle in suggested by the increased VA conduction time and the increased cycle length of SVT

lead standard I. A negative P wave in lead I during SVT can be detected in tachycardias with retrograde bypass tract conduction but not in patients with AV-nodal re-entry tachycardia /4/. Unfortunately, we did not notice this important electrocardiographic sign earlier but retrospectively with the help of intracardiac recordings negative P wave on standard lead of I could be suggested (Fig. 5).

3. Association of functional BBB with AV-junctional re-entry tachycardia is rare (8.0%), while the incidence of BBB in concealed W.-P.-W. syndrome is more frequent (66.0%) /12/.

4. The high rate of SVT in our presented case also suggested the existence of concealed W.-P.-W. syndrome. The average rate of SVT involving a concealed accessory pathway is faster than of AV-nodal re-entry tachycardia.

5. Taking into consideration the age, sex, no evidence of heart disease can be supposed that concealed Kent bundle was responsible in our patient for the recurrent SVT.

Clinical implications

Knowledge about the exact mechanism of a supraventricular tachycardia with wide QRS is extremely important for treatment and prevention of the arrhythmia. In a given patient with wide QRS tachycardia we must establish the origine of the tachycardia. In our presented case several electrocardiographic and electrophysiologic characteristics of the tachycardia excluded the existence of ventricular tachycardia. There was no evidence of atrioventricular dissociation, capture, fusion beats and H-potentials preceeded V waves during tachycardia.

Further differential diagnostic possibilities are the followings: SVT with preexistent BBB, AV-junctional re-entry tachycardia with aberrant ventricular conduction, W.-P.-W. syndrome with antidrom tachycardia and concealed W.-P.-W. syndrome with functional BBB during tachycardia. The diagnosis of a concealed accessory pathway can be supposed if the increase of cycle length during the tachycardia associated with the appearance of functional BBB /2, 11/. The location of concealed anomalous pathway can be suspected ipsilateral to the BBB configuration (left or right). The demonstration and explanation of this can be seen in our case in Figure 8. Occurrence of RBBB had no effect on cycle length and retrograde VA conduction time during SVT. However the cycle length and VA conduction time during SVT with LBBB were longer than during SVT with a normal QRS complex or with RBBB. The presence of LBBB during tachycardia necessitates the supposition that the anterograde ventricular activation will first pass through the right ventricle and than slowly by intraventricular conduction reach the concealed bypass tract in the left ventricle. It is obvious that the slower ventricular conduction lengthens the re-entry circuit therefore tachycardia cycle length and retrograde VA conduction will be prolonged. Therapeutic approach of SVT with concealed accessory pathway might be different from those with junctional re-entry tachycardias. The administration of drugs depressing the AV-node (beta-blockers, verapamil) allows a given extrastimulus to cause greater AV-node delay which delay potentiates the concealed retrograde conduction through anomalous pathway because it is given more time for recovery from antegrade concealed penetration. Thus drugs, as procainamid, quinidine, amiodarone should be used in these circumstances which depress anterograde pathway conduction and likely prolong retrograde refractoriness of the concealed accessory pathway, therefore preventing of maintenance of re-entrant SVT /21/.

In patients with drug-resistant tachycardias due to the above-mentioned mechanism, invasive diagnostic methods such as endocardial mapping and surgical intervention should be considered /18/. A curative therapeutic alternative to surgery has recently offered the catheter ablation method. Ablation of these accessory pathways can be achieved by application of catheter induced radiofrequency current with high efficacy and safe /7, 9/. The exact localization of the accessory pathway is the most important prerequisite for a successful catheter ablation and surgical intervention /6, 9/.

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Acta Medica Hungarica, Vol. 50/1—2, pp. 55—64 (1994)

PATHOPHYSIOLOGICAL ASPECTS OF THE PROTECTIVE EFFECT OF MAGNESIUM IN MYOCARDIAL INFARCTION (REVIEW)

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(Received: January 10, 1994)

Intravenous administration of magnesium has proved to have beneficial effect in acute myocardial infarction. Magnesium seems to act at different levels of the cardiovascular system. Of the greatest importance is the direct influence of Mg^{2+} on the cardiomyocyte which includes: reduction of cytoplasmatic calcium overload, protection of mitochondria against calcium influx, and diminution of cellular potassium, magnesium and ATP depletion. By means of these effects, or by its direct action on myocardium, Mg^{2+} inhibits the origin of postinfarctional dysrhythmias. Furthermore, magnesium reduces afterload by decrease in vascular resistance, and improves coronary flow.

The mechanism underlying the protective effect of magnesium remains complex and poorly understood. Nevertheless, Mg^{2+} therapy is effective, undemanding, and easy to procure. Expectably, intravenous administration of Mg^{2+} may become a routine part of myocardial protection in acute myocardial infarction.

Keywords: Magnesium, myocardial infarction, myocardial protection

Introduction

Contemporary cardiology continues to expand therapeutic approaches to reduce the extent of damage caused by myocardial infarction (MI). Besides invasive interventions a good number of pharmacological preparations are used, e.g. beta blockers, aspirin, and trombolytics /19/. In the last decade Mg^{2+} has been frequently used as a therapeutic agent against MI. However, the first experimental data on protective effect of Mg^{2+} against myocardial infarction have been achieved several decades ago /9/ and protective effect of magnesium on the cardiovascular system has been empirically utilised over the last 50 years /13/.

Abbreviation: MI: myocardial infarction

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Surveys presenting several controlled clinical trials /29, 43/ suggest positive effect of intravenously applied Mg^{2+} during MI. Ionized magnesium was shown to reduce the incidence of dysrhythmias /1, 34, 38, 41/ and cardiac shock /34/. Rassmussen et al. /34/, Smith /41/ and Shechter et al. /38/ observed a substantial decrease in mortality in magnesium treated groups in comparison to placebo-treated groups. However, a comparison between these trials is made difficult because of different doses of magnesium administered, different duration of therapy and lack of uniformity of the criteria.

The LIMIT-II trial (the second Leicester Intravenous Magnesium Interventional trial) represent by far the largest experience with magnesium in acute MI /51/. This single-centre study randomised 2300 patients with suspected acute MI to receive intravenous $MgSO_4$ with 8 mmol given as a bolus over 5 min and then 65 mmol given as an infusion over 24 h, or a matching placebo infusion. At 28 days, the relative reduction of mortality was 24% in magnesium-treated group in comparison to placebo-treated group and incidence of left ventricular failure was decreased in 25% /51/. The beneficial effect of intravenous Mg^{2+} treatment lies probably in the acute phase. However, the benefit attained here was not lost during the following year /33/.

Despite these undoubtedly promising clinical data the mechanism of magnesium protective effect for MI is still unclear and remains a topic of contraversy /44/.

Compartmentalization of Mg^{2+} in the body under physiological conditions and in the course of MI

Magnesium is the second most prevalent ionic species of the intracellular milieu. Out of its total content in the human body 60% is within the bone matrix, 39% in the intracellular space and only 1% is extracellular /46/. Its concentration in the myocardial cell is about 17 mM/kg of the cellular fluid /30/. A major fraction of cellular magnesium is presumably present as Mg complexes such as adenosine triphosphate /ATP/ and other adenosine nucleotides. Because most of the adenosine nucleotides are supposed to be associated with the myofibrils, contractile proteins represent the richest cellular compartment of bound magnesium /30/. However, for the majority of biochemical processes the level of free ionised magnesium (Mg^{2+}) is decisive. The intracellular concentration of Mg^{2+} is about 1.0 mM /49/.

Extracellular magnesium concentration under physiological conditions lies between 0.75 and 1.2 mM/l /8/. In the course of acute MI, a rapid increase of serum magnesium concentration takes place at the onset. Very quickly it is decreased to below normal values, and within 36-48 h the serum magnesium concentration reaches normal values again /17, 29/.

The early magnesaemia increase, which appears before the creatinkinase increases, is probably caused by elimination of magnesium from the ischaemic myocardium /17/. Immediately after the onset of infarction ATP is split, magnesium is released from the ATP complex and as a result cytoplasmatic magnesium ion concentration increases transiently /18/. The increase in the concentration gradient of Mg^{2+} across the cardiomyocyte membrane and the deterioration of the sarcolemma by ischaemia may both be responsible for leakage of magnesium ions from the cardiac tissue /18/. This assumption is supported by the finding of decreased magnesium concentration in the myocardium of patients who died of ischaemic heart disease /14/.

The subsequent decrease of Mg^{2+} serum concentration is supposed to be caused by postinfarctional sympathetic stimulation. The increased sympathetic tone stimulates postinfarctional diuresis which possibly results in the loss of magnesium in the urine /17/ and augments lipolysis with subsequent binding of magnesium with free fatty acids in adipocytes /15/. Hypomagnesaemia is not specific for postinfarctional sympathicotonia, but also occurs during other types of stress /4/.

The return of Mg^{2+} to normal levels is likely mediated by the kidney which represents the main regulator of magnesium homeostasis. Excretion of magnesium responds directly to the load presented to the kidney-high serum magnesium levels lead to diminished renal tubular reabsorption and vice versa /24/.

The physiologic role of magnesium in the cardiomyocyte

Both intra- and extracellular magnesium participate in many processes within cardiomyocyte including contraction, enzyme activation and ion transport processes /30, 39, 49/, while interactions of Mg^{2+} with Ca^{2+} and K^{+} are supposed to be of greatest physiological importance.

Magnesium--calcium interactions

Mg^{2+} antagonizes the effect of Ca^{2+} ions at different levels of the heart muscle cell. Mg^{2+} inhibits the slow calcium channel, sometimes considered to be the decisive component of the calcium-antagonistic effect of Mg^{2+}. This effect is more pronounced when the channel is activated /49/. Mg^{2+} has, however, a relatively low affinity to the calcium channel and causes only a mild blockade /23/. Mg^{2+} is by three to five orders of magnitude less potent than the organic Ca^{2+} channel blockers, e.g. verapamil or nitrendipine /5/ but, compared to these compounds, a distinct advantage of Mg^{2+} is its broad spectral effects it has on volatage-, receptor- and leak-oparated membrane channels /5/. It seems therefore, that Mg^{2+} in its physiological concentration may have a role as a non-specific natural cardiac and vascular blocker of the slow calcium channel /20/.

The level of intracellular Ca^{2+} is also influenced through interactions between magnesium and the sarcoplasmic reticulum /SR/. Mg^{2+} is the cofactor of SR Ca^{2+}-ATPase /10/ and a normal level of cellular Mg^{2+} is essential for the activity of this enzyme, so permitting active uptake of Ca^{2+} with the subsequent relaxation /49/. Magnesium also blocks calcium induced calcium release by inhibition of propagation of this process along the intracellular network of the cardiomyocyte's sarcoplasmic reticulum /16/.

Besides regulation of the cytoplasmatic Ca^{2+} concentration, Mg^{2+} influences the cardiomyocite's calcium action in several other ways. Mg^{2+} was shown to reduce the uptake of Ca^{2+} by mitochondria, which would be deleterious to oxidative phosphorylation /20/. Moreover, free magnesium ions participate in modulating tension development in cardiac tissue. A plausible mechanism may be the interaction of Mg^{2+} and Ca^{2+} ions on the non-specific sites of troponin C, which constitutes a locus of direct competition between ions of calcium and magnesium at the level of the contractile proteins /31/. The final level of conformation changes on troponin C, and thus also the degree of derepression of actino-myosin interactions on which contraction intensity depends, may be determined by the relation of Ca^{2+} to Mg^{2+} ion concentrations during systole /24/.

Effects of magnesium on potassium metabolism

There is a good evidence suggesting that Mg^{2+} and K^{+} metabolism is closely linked. It was, e.g., shown that an increased magnesium concentra-

tion in the perfusion fluid prevented potassium release from the isolated myocardium /40/. It was also revealed that in some patients with hypokalaemia the repletion of potassium alone failed to normalize the serum potassium level. This potassium-refractory hypokalaemia is caused by concomitant magnesium deficiency, and can be corrected with magnesium supplementation /47/. The relative representation of Mg^{2+} and K^{+} in lymphocytes may be a prognostic factor of dysrhythmia development /2/ and mortality /3/ in patients with acute MI.

The mechanism of Mg^{2+} and K^{+} interaction in the cardiomyocyte is not fully understood. Although Mg^{2+} acts as Na^{+}-K^{+}-ATPase cofactor /42/, the deficit of this ion does not seem to significantly affect enzyme activity /11, 39/. Of greater importance seems to be that Mg^{2+} may determine the level of cellular K^{+} by acting on potassium channels. Several of the K^{+} channels exhibit inward rectification, i.e. they allow K^{+} to pass more readily in the inward direction than in the outward direction /25/. It appears that this inward rectification may be caused by internal Mg^{2+} blocking the outward movement of K^{+} through these channels. In the absence of magnesium, transport in both directions takes place at the same intensity /25/.

Possible mechanisms of Mg^{2+} beneficial effect during MI

Mg^{2+} protective effect within the cardiomyocyte

It is not clear and needs further study, which of the above-mentioned mechanisms might result in protection against MI /44/. However, knowing the mechanism of deleterious effect of ischaemia it is supposed that during MI Mg^{2+} may reduce cytoplasmatic calcium overload and protect the mitochondria against calcium influx /52/. Intravenous application of magnesium is supposed to prevent cellular depletion of Mg^{2+} /8, 18/. Through obstructing effect upon the potassium channel Mg^{2+} might help to lower the potassium loss /49/. Moreover, magnesium is necessary for Mg-ATP regeneration /18/ and decreases high-energy phosphate depletion during MI /8/.

Antiarrhythmic action of magnesium

The antiarrhythmic effect of magnesium if one of its earliest clinically utilized properties /13/. Already in the sixties the treatment with

K^+-Mg^{2+} asparaginic acid was shown to reduce the incidence of dysrhythmias in patients with various cardiovascular diseases /6, 7/. Magnesium reduces the incidence of supraventricular /35/ as well as the potentially lethal dysrhythmias that occur during acute MI /1, 41/. Elevation of extracellular magnesium levels prolong conduction time and the refractory period in atria and AV node /33/. On the other hand, decreased levels of extracellular Mg^{2+} shorten the effective refractory period, but lengthen the relative refractory period, thereby increasing the vulnerability of the ventricles to fibrillation /45/. The antiarrhythmic properties of Mg^{2+} depend heavily on the concentration of other ions, particularly of potassium /45/ and are mediated either by a direct effect of magnesium on myocardium, or by its calcium-antagonistic affect and interaction with potassium /46/.

Effects of Mg^{2+} on systematic and coronary circulation

A number of authors believe that mortality reduction as a result of intravenous administration of Mg^{2+} in MI is not a result of its antiarrhythmic effect because dysrhythmias after infarction are successfully affected by aimed antiarrhythmic therapy /38, 44/. Larger importance is attributed to the positive haemodynamic effect of magnesium including an inhibition of the myogenic, basal and hormonal-induced tone of vascular smooth muscule in the large vessels, arterioles and venules in all regional vasculatures so far examined /2/. This action is supposed to be determined by Mg^{2+} ability to inhibit the slow calcium channel /2/ and/or by stimulation of prostacyclin release /28/. The next effect is a decreased blood pressure as a result of the peripheral arterial resistance while the contractility and the frequency of the heart are not affected /36/. Magnesium has also an advantageous effect on coronary circulation. It inhibits tonic contraction of coronary spasm as a consequence of physical load /22/ or hyperventilation /27/ as observed in patients with variant angina pectoris. Reduction of afterload and improvement of coronary blood flow may both improve the relationship between oxygen supply and consumption during MI /36/.

The main role of the Mg^{2+} protective effect during acute MI seems to be determined by its direct action on the cardiomyocyte /51, 52/. Reducing afterload, improvement of coronary flow and limitation of dysrhythmias are also of importance /33/. The presumed antiaggregation /12/ and anticoagulation potential /33/ of Mg^{2+} may play a supportive role.

Questions to be answered

In connection with intravenous administration of Mg^{2+} during MI several questions still remain unanswered:

— Should Mg^{2+} be administered in suspected acute MI only in cases of evident hypomagnaesemia, or in every case (since serum levels of Mg^{2+} do not reflect the intracellular concentration of this ion) /29, 37, 47/?

— Is the therapy's goal to normalize serum Mg^{2+} levels, or to prevent its postinfarctional decrease or to achieve its slight increase to above normal values /50/?

— Taking into account that hypomagnaesemia is considered a risk factor for sudden death /32/, mortality in ischaemic heart disease /14/, as well as in other cardiovascular diseases /37/, should determination of magnesium level and its theraputic correction become part of a routine diagnostic and therapeutic practice /47/, especially in patients with cardiovascular diseases /37/?

The mechanism of protective effect of magnesium is complex, poorly understood and thus more or less hypothetical at the present time. Again it appears that we are facing the old problem of not understanding the mechanisms of action of an administered drug. However, the clinical results of venous application of magnesium are very promising. Moreover, Mg^{2+} therapy is simple, inexpensive, of limited side effects and non-apparent potential to interact with other drugs. These indisputable preferences enable the use of this substance for a broad spectrum of therapeutic care /26, 29, 41, 52/. The mentioned facts suggest that venous administration of magnesium may become a routine part of myocardial protection in acute infarction in the near future.

Acknowledgements: This work has been written during the research stay of the author at the "Zentrum Physiologie und Pathophysiologie" in Göttingen, under the support of Alexander von Humboldt Foundation, Bonn—Bad Godesberg.

The author expresses his thanks to Julian Paton, PhD. for suggestions to the manuscript and improving its English style.

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Acta Medica Hungarica, Vol. 50/1—2, pp. 65—74 (1994)

METABOLISM

POLYMORPHIC PARACETAMOL CONJUGATION: PHENOTYPING IN A HUNGARIAN POPULATION

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(Received: December 28, 1993)

The authors studied the distribution of the paracetamol conjugation in a Hungarian population (53 adult Caucasian persons). The data indicated that the excretion of paracetamol glucuronide and sulphate were not normally distributed. Bimodality were apparent in both conjugation pathways: 15.1% of subjects was relatively extensive glucuronidators, and the 24.5% of subjects was extensive sulphatators. Monitoring the ratios of various urinary paracetamol conjugates/paracetamol may be useful as a tool for determining the glucuronide and sulphate conjugation capacity in humans.

Keywords: Paracetamol, HPLC, glucuronidation, sulphatation, conjugation capacity, drug conjugation

Introduction

Paracetamol (acetaminophen) is excreted as glucuronide (55 to 60% of the dose administered) and sulphate (35% of the dose administered) conjugate from the human body and only a very small amount undergoes the oxidative metabolic route via the cytochrome P-450 system leading to formation of a reactive intermediary, the N-hydroxy-N-acetyl-4-aminophenol conjugated then with glutathione. Trace amounts of conjugates with cystein and mercapturic

Abbreviations: UDGPT — Uridinediphosphoglucuronosyl transferase, PST — phenyl sulphatetransferase, M/P — metabolite/paracetamol ratio, P — paracetamol, G, S — glucuronide and sulphate conjugated paracetamol

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0236-5286/94/$ 4.00

acid conjugates can also be detected. Only 3% of the administered paracetamol dose is excreted unchanged. Paracetamol is therefore a good test agent for studies in phase II (conjugative) metabolism /2/.

Uridinediphosphoglucuronosyl transferase (UDGPT) is readily inducible by phenobarbital and methylcholantrene type enzyme inducers, but this holds not true in the case of sulphate conjugation. The conjugation capacity of the individual can be characterized by the metabolite/paracetamol ratio (M/P), the ratio being 18 for glucuronide and 12 for sulphate conjugates in healthy volunteers /3/. Phenytoin or rifampicin pretreatment results in an increase to 41 and 35, respectively, in the ratio of the glucuronide formation. Smoking of 40 cigarettes a day leads to an induction in a glucuronide formation equivalent to rifampicin administration, but only to a minimal induction of sulphate conjugation. There was a 17% increase in paracetamol glucuronidation in volunteers on a 10-day Brussels sprouts and cabbage diet compared to controls and no significant change in sulphate conjugation /12/.

The two key transferases in paracetamol conjugation differ in localization, too, UDGPT being prevalent in the endoplasmic reticulum /4/ and sulphatetransferase (PST) in the cytosol of hepatic cells /5/.

There is a significant decline in sulphate conjugation without any change in glucuronide formation in patients suffering from Parkinson's disease and various motoric function losses /17/. Similar results were obtained from a population of patients with rheumatoid arthrosis. A weak-S-carboxymethyl-cysteine sulphoxidation may be responsible for this phenomenon.

However there is no difference in paracetamol conjugation in Gilbert's disease compared to healthy controls /20/. The bilirubin conjugation is impaired in Gilbert's disease and this may imply a possible existence of various UDPGT isozymes /3/. Paracetamol half life is increased and the glucuronide and sulphate conjugation rates are diminished in acute and chronic liver diseases /5/. There is an increase in glucuronide conjugation in obese patients /1/.

Glucuronidation is increased by 75%, the oxidation by 88% in pregnant women without any change in sulphate conjugation /9/. Anticonceptives have similar effects /4/.

Age seems to play no major role in the sulphatation capacity but there is a 23% decline in glucuronide formation capacity in the population above the age of 80 years compared to the population of the age of 20 /10/.

Glucuronidation is absent or inefficient in newborn babies and young children /13/, paracetamol half life is also prolonged, and the main metabolic route is sulphate conjugation. This finding is, however, disputed /6/.

Sulphate conjugation of paracetamol is a saturable process. The limiting factor is the sulphate pool available /8/. The rapid sulphate conjugation rate drops to half activity after 4 h, glucuronidation reaches its steady-state level after 6 h /13/.

The interindividual and interethnic differences of paracetamol conjugation and the genetic background (twin studies) confirmed the monogenetic regulation of the UDGPT and PST activities in man /4, 18, 21/. A 300% interindividual difference in paracetamol glucuronide and sulphate conjugate formation was demonstrated in twins /11/. The differences were grater in monozygote twins hinting at the dominant impact of environmental factors over the genetic ones. On the contrary, in another study there were no significant differences in the metabolic pattern of paracetamol between twins of the Venda tribe living in South Africa and in Europe /15, 16/. Significant interindividual differences in UDGPT /4/ and PST activities /21/ were also reported. The urinary M/P ratio had 27% RSD according to Bock /2/, while this value was only 14% when repeated on the same subject.

The controversies in the data generated by previous studies motivated us to carry out a study on paracetamol glucuronide and sulphate conjugation capacity distribution in a Hungarian population.

We have developed a liquid chromatographic method /14/ for determination of paracetamol and its glucuronide and sulphate conjugates from urine. After oral paracetamol administration we determined first the excreted unchanged ("free") paracetamol (P), then the enzyme-pretreated samples were further analysed for the glucuronide (G)- and sulphate (S)-conjugated paracetamol. The G/P and S/P ratios were calculated as representative values for individual conjugation capacity.

Subjects and Methods

After giving their informed consent 53 healthy volunteers (mean age 37.5 $\pm$ 14.3, 38 women and 15 men) participated in the study. All of them were nonsmokers. The subjects swallowed 500 mg paracetamol (1 tablet of Rubophen, Chinoin Chemical Works, Budapest, Hungary) with 1 dl tap water while fasting in the morning, and collected their urine for the consecutive 8 h. These urine samples were stored at -20 ^{o}C until further analysed.

The unchanged ("free") paracetamol was determined in the first step by liquid chromatography (internal standard: 3-acetaminophenol). The urine samples were pretreated with beta-

glucuronidase and the "total" (free and conjugated) paracetamol was determined in the second step. This method detects, beside the "free" paracetamol, the glucuronide- and sulphate-conjugated paracetamol after the beta-glucuronidase/sulphatase cleavage.

The paracetamol content of the glucuronide (G) and sulphate (S) conjugate was calculated from the paracetamol (P) measured by subtraction and a metabolite/paracetamol (M/P) ratio was formed. The "free" (unconjugated) paracetamol was extracted by adding 50 µg 3-acetminophenol internal standard (1 mg/ml) to 1 ml of urine sample and adjusting its pH to 10 with 0.25 M sodium hydroxide. After addition of 8 ml ethylacetate the samples were shaken mechanically for 10 min. The upper layer was separated, filtered through 1 PS phaseseparator (Whatman), and dried under nitrogen stream. The dry residues were made up in 100 µl of methanol:water (3:7, v/v) mixture: 20 µl volumes were injected.

The "total" paracetamol was extracted by adding 1 ml of 0.2 M (pH = 5) acetate buffer and 50 µl of beta-glucuronidase/arylsulphatase or only beta-glucuronidase (Serva) and 500 µg of 3-acetaminophenol internal standard to 1 ml of urine sample. After mechanic shake the samples were incubated in a 37 ^{o}C water bath for 2 h. The pH of the samples was adjusted to 10 with 1 ml of 0.25 M sodium hydroxide after the incubation period. 8 ml of ethyl-acetate was added followed by a 10 min shaking and the separation of the upper layer. The upper layer was filtered through 1 PS phaseseparator (Whatman) and dried under nitrogen stream. The residues were made up in 1000 µl of methanol:water (3:7, v/v) mixture; 20 ul volumes were injected. Beckman 110B pump, Labor MIM 308 UV detector, Rheodyne 7125 injector with 20 µl loop and Shimadzu C-R6A integrator constituted the liquid chromatographic system. Separation was carried out at room temperature on Spherisorb-5-ODS (250 x 4.6 mm I.D., Chrompack) column. The mobile phase was methanol:water (3:7, v/v) mixture, the flow speed was 1 ml/min. The monitoring wavelength was 254 nm.

Results

The excreted amounts of free paracetamol (P), its glucuronide (G) and sulphate (S) conjugates in proportion to the dose administered (500 mg) are shown on Table I. The G/P ratios are shown in Fig. 1. The cumulative frequencies of the S/P ratios are represented in Fig. 2.

The normal distribution pattern of the M/P ratios could be rejected both in the case of G/P and S/P ratios (95% confidence interval, goodness of fit test 5.1 $\pm$ 4.2 S.D. for G/P and 15.7 $\pm$ 14.5 S.D. for S/P, χ^2 = 33.18 for G/P and 38.62 for S/P). The population could be divided into two distinct populations by the antimode of the cumulative M/P ratio frequencies (Table

Table I

Eight hour urine-excrected paracetamol conjugates in healthy volunteers (n=53)

	Free paracetamol (dose%)	Paracetamol glucuronide (dose%)	Paracetamol sulphate (dose%)
mean ($\bar{x}$)	2.98	7.74	21.67
$\pm$ S.D.	2.47	7.3	13.76

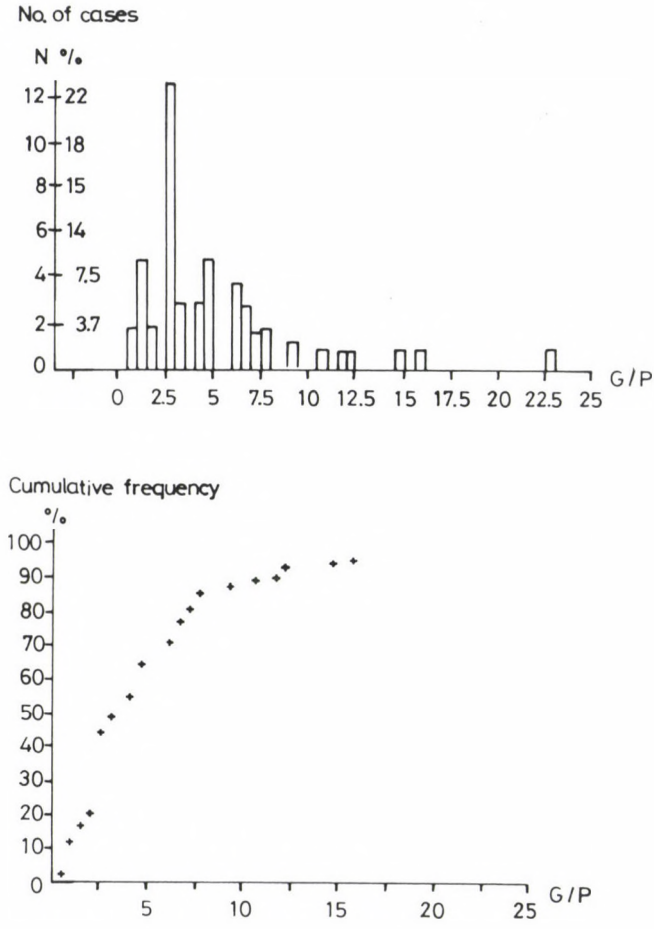

Fig. 1. The distribution pattern of G/P

II). The "normal conjugator" population has a G/P ratio < 8 and and S/P ratio < 18, while the "intensive conjugators" have a G/P ratio > 8 and an S/P ratio > 18 (Table III).

The correlation coefficient between G/P and S/P was 0.379. We found 2 normal sulphate conjugators out of the 8 intensive glucuronidators and 7 normal glucuronidators out of the 13 intensive sulphate conjugators.

No correlation was found between age and conjugator capacity (M/P). The correlation coefficient was 0.020 for G/P and 0.091 for S/P.

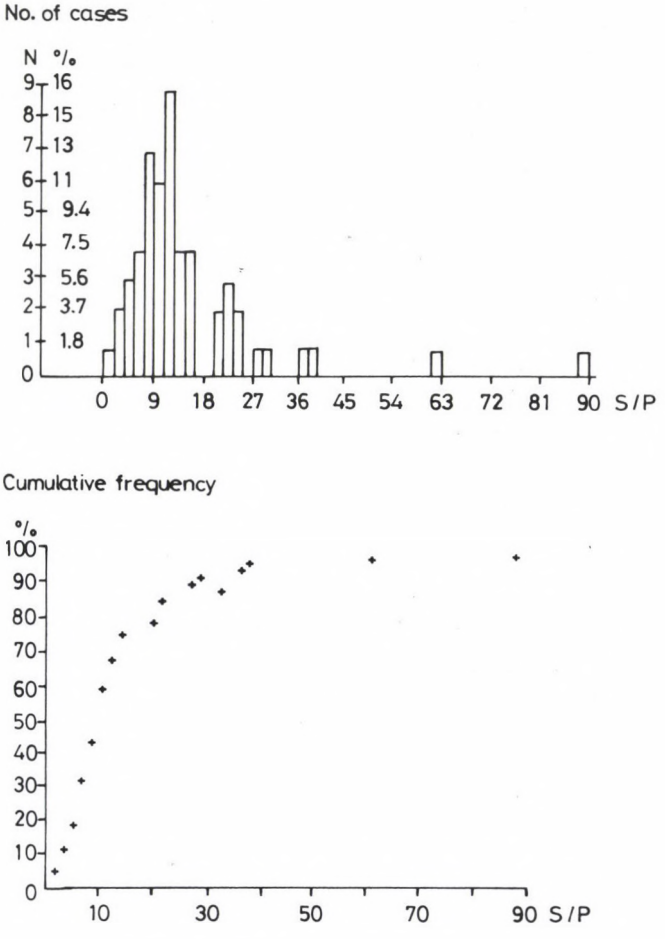

Fig. 2. The distribution pattern of S/P

Discussion

With regard to the chromatographic characteristics of the analytical method for the investigation of paracetamol conjugation elaborated by us we refer to our previous papers /14/.

We demonstrate in Fig. 3 a chromatogram showing the separation of 4-acetaminophenol (paracetamol) from 3-acetaminophenol (internal standard) and 2-acetaminophenol. The separation factor (α) of the internal standard and paracetamol was 2.59. The typical blank (A) and an after-treatment (B) urine sample extract chromatogram is shown in Fig. 4.

Table II

Distribution of paracetamol conjugation in the Hungarian adult population (n=53)

	Glucuronide conjugation				Sulphate conjugation			
	G/P < 8		G/P > 8		S/P < 8		S/P > 8	
	n	%	n	%	n	%	n	%
No. of cases (n=53)	45	84.9	8	15.1	40	75.5	13	24.5
Men (n=15)	12	80	3	20	12	80	3	20
Women (n=38)	33	86.8	5	13.2	28	73.7	10	26.3

P — paracetamol; G — glucuronide conjugated paracetamol; S — sulphate conjugated paracetamol

Table III

Conjugation capacity of the Hungarian population (n=53) according to sex

	M/P ± S.D.		
	Total population	"NC" phenotype	"IC" phenotype
Glucuronidation (G/P)			
Men	6.1 ± 6.1	3.6 ± 2.3	16.3 ± 6.1
Women	4.6 ± 3.1	3.7 ± 1.9	11.1 ± 2.7
Significance	t = 1.106 (N.S.)	t = 0.194 (N.S.)	t = 1.678 (N.S.)
Sulphatation (S/P)			
Men	13.3 ± 7.9	9.9 ± 4.1	26.6 ± 3.9
Women	16.7 ± 16.4	9.8 ± 3.3	35.9 ± 22.7
Significance	t = 0.785 (N.S.)	t = 0.021 (N.S.)	t = 0.686 (N.S.)

M/P — metabolite/paracetamol

There is a correlation (r = 0.84) between the paracetamol clearance and the volume of the collected urine /7/. We investigated only those urine samples the collected volume of which was between 150 and 800 ml. We did not find a correlation between the M/P ratios under these circumstances (r = = 0.107) and this is in good accordance with the results of Bock et al. /2/. The urinary pH did not influence the results obtained.

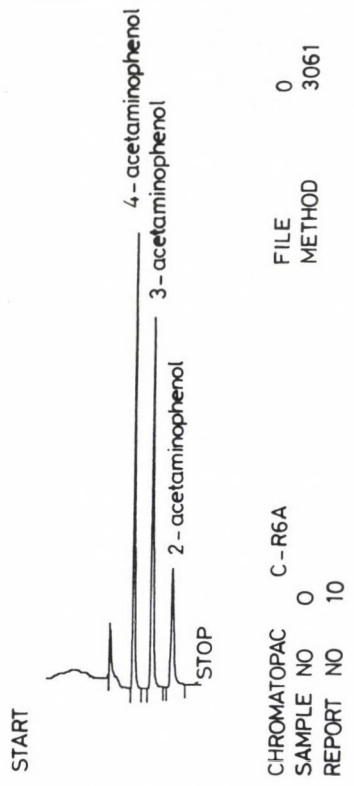

Fig. 3. Chromatographic separations of acetaminophenols. Chromatographic conditions in the text

The effect of smoking was not taken into account, for all our voluntereers were nonsmokers.

There was no correlation between the glucuronide (G/P) and sulphate (S/P) conjugation, what can be explained on grounds of different localization, inducibility and other biochemical differences between the two transferases /4, 21/. Both M/P distributions contradict the normal distribution pattern (x^2-test, 95% probability level).

The variation coefficient for G/P was 82.7% ($x \pm S.D. = 5.08 \pm 4.2$) and for S/P 91.5% ($x \pm S.D. = 15.7 \pm 14.5$). These data allow the distinction between two phenotypes with regard to glucuronidation and sulphatation within the population. 15.1% were extensive glucuronidators and 24.5% were extensive sulphate conjugators in the Hungarian population studied. Age and gender had no significant effect on phenotype ($p > 0.05$, N.S.).

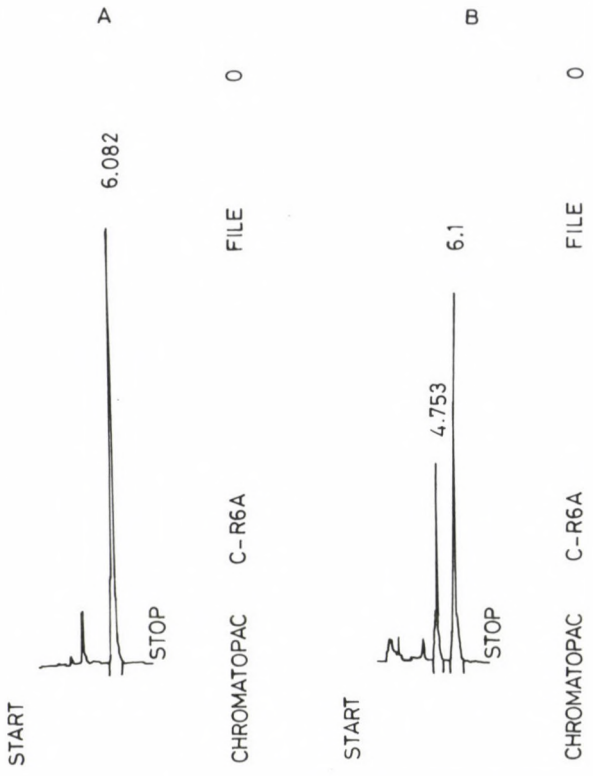

Fig. 4. Chromatograms of urine extracts (A) blank with the internal standard, (B) typical sample. Chromatographic conditions in the text

The gene frequences for UDGPT were p = 0.612, q = 0.388 and for PST p = 0.506, q = 0.494 (calculated according to the Hardy—Weinberg law).

The glucuronide formation and sulphatation play a key role in the "end phase" of drug metabolism. The insufficiency of the monogenically determined UDPGT and PST activities is manifested in Gilbert's syndrome, Crigler—Najjar's disease, certain chronic neurologic disease states (e.g.: Parkinson's disease) and rheumatoid arthritis /17, 18, 20/. Paracetamol is a suitable test agent for conjugation capacity determinations /19/.

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Acta Medica Hungarica, Vol. 50/1—2, pp. 75—81 (1994)

SERUM CHOLESTEROL PROFILE OF SOME NIGERIAN PREGNANT WOMEN

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(Received: December 30, 1993)

A semi-longitudinal study of the cholesterol profiles at various stages of pregnancy was conducted. The study involved 49 pregnant women who showed no physical signs of obesity, were neither hypertensive nor diabetic, and had a mean age of 24.7 ± 4.5 (mean ± SD). The results showed a progressive increase in the serum total and high-density lipoprotein (HDL) cholesterol levels from 4.02 ± 0.39 mmol/L (mean ± SD) and 1.81 ± 0.15 mmol/L, respectively, at 3 months, to 5.59 ± 0.51 mmol/L and 2.46 ± 0.18 mmol/ in the ninth month of pregnancy. These represent a 39 ± 11% and a 35 ± 10% increase in total and HDL cholesterol, respectively, over the 3-month level. The levels of total and HDL cholesterol however decreased to 4.08 ± 0.40 mmol/L and 1.89 ± 0.17 mmol/L, respectively, a month after delivery. The most significant ($P < 0.05$) month to month increase was recorded between the 6th and the 7th month for both total and HDL cholesterol. The proportion of HDL cholesterol remained fairly constant at 43 ± 3 to 45 ± 4% throughout the period of pregnancy covered, and one month after delivery. This suggests a proportional increase in all lipoprotein fractions. It is concluded that the observed changes are normal physiological events.

Keywords: Pregnant women, gestational ages, total cholesterol, HDL cholesterol

Introduction

Plasma lipids are associated with macromolecular complexes known as lipoproteins. Total cholesterol is a measure of the cholesterol content of all lipoproteins. Because the cholesterol composition of lipoproteins is fairly constant, the cholesterol content of the various lipoproteins is often used as a measure of their concentration in the plasma /2/.

Abbreviations: HDL — High Density Lipoprotein, LDL — Low Density Lipoprotein, VLDL — Very Low Density Lipoprotein

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Changes in plasma cholesterol may be due to disease /8, 10, 11/, diet /6, 12/, age and other physiological changes involving abnormalities of sex hormones /9/. Pathological chances in cholesterol levels usually involve the lowering of HDL cholesterol level and increases in total, low density lipoprotein (LDL), and very low density lipoprotein (VLDL) cholesterol /3/. Such changes are known to correlate positively with the development of severe, and premature atherosclerosis /4, 7/ and coronary heart disease /5/.

Pregnancy is a stressful physiological state during which hormonal changes are induced. Some of the involved hormones affect lipid metabolism /15/. The aim of this study is to determine the serum cholesterol profile of some Nigerian women at different stages of pregnancy in a semi-longitudinal manner and to compare the HDL/total cholesterol ratio at each stage. Although cholesterol levels in some pregnant women had earlier been carried out /13/, to our knowledge, no longitudinal study has been conducted on Nigerian women.

We believe that the findings in this study will enable the clinician to differentiate between lipid changes due to pregnancy per se and those due to possible complications of pregnancy or other diseases which may occur during pregnancy.

Pregnant women and Methods

Subjects: The following criteria were used for the selection of subjects for the study.
Blood pressure: Systolic 120 mmHg or less
Diastolic 70 mmHg or less
Fasting blood glucose — 3.3.-4.5 mmol/L.
Urine glucose: Nill
Age: 22—30 years
Habitus: Lean — no tendency to obesity.

No previous history of diabetes or hypertension. There was however no control on diet.

The study was started with 28 pregnant women who reported at the Maternity Clinic of the University of Calabar Teaching Hospital with 3 month old pregnancies. More subjects were incorporated into the study at gestational ages of 4, 5 and 6 months, respectively, at which time the number of participants rose to 49. All subjects remained in the study to the end had normal deliveries.

Sample collection and analysis

Fasting blood samples were collected once a month during visits to the clinic. Samples were also collected from the same subjects in the postnatal clinic one month after delivery. Sera separated from the samples so collected were analysed for total and HDL cholesterol within 24 h.

Total and HDL cholesterol estimation

Cholesterol estimation was performed according to the method of Bachorick et al. /1/. For total cholesterol 0.10 ml of serum was extracted with 2.4 ml of chloroform/methanol (2:1 v/v). 1.0 ml of the supernatant from the extract was mixed with 0.06 ml of 2.5% ferric chloride and 1.0 ml of glacial acetic acid. Two millilitres of concentrated sulphuric were then added and thoroughly mixed. The resulting coloured solution was examined at 550 nm in a Shimadzu UV 120-02 spectrophotometer.

Two standards (2.5 mmol/L and 5 mmol/L) and Sigma control sera (normal and abnormal range) were analysed along with the test samples. The absorbance of standards was used to calculate the values of the test and controls.

HDL cholesterol

This fraction was separated from the other lipoproteins by the Heparin manganous chloride method described by Burnstein and Samaille /2/. The supernatant, which contained the HDL, was analysed for HDL cholesterol as described above for total cholesterol except that instead of 0.1 ml, 0.2 ml of the supernatant was extracted with chloroform/methanol (2:1 v/v).

Statistical analysis

The significance of changes in lipid profile at various ages of gestation was assessed by the one-factor analysis of variance while the significance of differences between any two ages of gestation was assessed by Student's t-test.

Results

The result of the pre-test screening for all subjects is shown in Table I.

Table I

Results of pre-test screening of subjects

Parameter		Value
Blood Pressure:	systolic	117 ± 2[+] mmHg
	diastolic	67 ± 4 mmHg
Fasting Blood Glucose		3.65 ± 0.23 mmol/l
Mean age		24.7 ± 4.5 years

[+]Mean ± SD

Cholesterol profile

A plot of the mean serum total and HDL cholesterol levels of participating subjects against gestational age is shown in Fig. 1. The graph

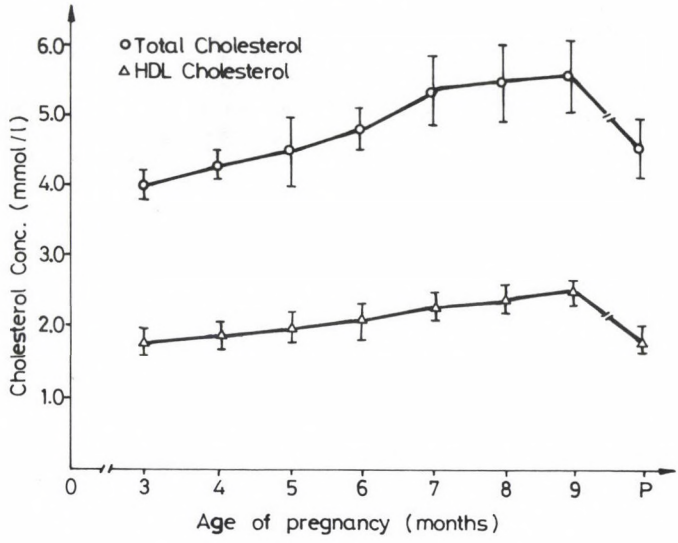

Fig. 1

shows a progressive increase in both the total and HDL cholesterol levels throughout the period of pregnancy studied. The highest monthly increases in both total and HDL cholesterol levels occurred between the 6th and the 7th months. The ninth-month levels of total and HDL cholesterol was 5.50 ± 0.51 and 2.46 ± 0.18, respectively (Table II). These levels were by $39 \pm 11\%$ and $35 \pm 10\%$, respectively, higher than the three-month levels. The proportion of HDL cholesterol to total cholesterol remained between $43 \pm 3\%$ and $45 \pm 4\%$ throughout pregnancy (Table I). One month after delivery the levels of both total and HDL cholesterol decreased significantly ($P < 0.05$) versus the ninth-month levels and were similar to those obtained in the third-month of pregnancy (Fig. 1). The percentage of HDL to total cholesterol was, however, not significantly altered ($P > 0.05$) a month after delivery.

Discussion

This study has shown that the levels of both serum total and HDL cholesterol increased with the age of pregnancy but the ratio of HDL cholesterol to total cholesterol remained constant. The constancy of the total/HDL cholesterol ratio may be an important differentiating factor between pathological and non-pathological increase in cholesterol levels, for

Table II

Serum total and HDL cholesterol levels at different ages of gestation

Parameters measured	Gestational age: months							**	Per cent increase 9th month vs 3rd month	4 weeks post-delivery	***
	3	4	5	6	7	8	9				
Total cholesterol (mmol/l)	4.02+ ± 0.39	4.25 ± 0.44	4.47 ± 0.49	4.84 ± 0.32	5.35 ± 0.52	5.51 ± 0.55	5.59 ± 0.51	$p < 0.05$	39 ± 11	4.08 ± 0.40	$P > 0.05$
HDL cholesterol (mmol/l)	1.81 ± 0.16	1.89 ± 0.18	2.03 ± 0.20	2.08 ± 0.20	2.32 ± 0.19	2.43 ± 0.19	2.46 ± 0.18	$p < 0.05$	35 ± 10	1.89 ± 0.17	$P > 0.05$
HDL cholesterol as % of total cholesterol	45 ± 4	44 ± 3	45 ± 3	43 ± 3	44 ± 4	44 ± 3	44 ± 3	$p > 0.05$		46 ± 4	$P > 0.05$
n =	28	31	41	49	41	28	29			18	

**Significance of difference between 9th and 3rd months levels; ***Significance of difference between post-delivery and 3rd month levels; mean ± SD

pathological increase in cholesterol level is always accompanied by a decrease in HDL cholesterol: total cholesterol ratio /3, 7/. An earlier study on diabetic patients under treatment showed that even when the serum total cholesterol levels were within the normal range, the HDL cholesterol levels were significantly reduced /14/.

An increase in cholesterol level at the full term of pregnancy was reported by Taylor et al. /13/, who attributed the increase to hormonal changes. It had also earlier been observed that women on estrogen had increased HDL cholesterol and reduced LDL cholesterol /15/. However, women who combine estrogen with progestins as oral contraceptives have normal levels of HDL and LDL cholesterol, while those on progestins alone have reduced HDL cholesterol /15/. In our study no significant change ($P > 0.05$) in the HDL cholesterol fraction was observed. This may be due to the fact that both estrogens and progestins are increased during pregnancy, thus, creating a similar situation to those of the women who combine estrogen and progestins as contraceptives /15/.

We suspect that the increase in cholesterol level during pregnancy may be important for the survival of the fetus, for the female sex hormones, such as estrogens and progestins, which are increased during pregnancy are formed from cholesterol. The cholesterol increase may therefore serve to meet the requirements for these hormones which are essential for fetoplacental well-being. The observed cholesterol changes should therefore be seen as a normal physiological event. It should also be noted that the normal levels of cholesterol in pregnant women would depend on the age of pregnancy and may rise up to a level exceeding by $39 \pm 11\%$ the levels of the non-pregnant women.

Acknowledgement: The authors are grateful to Professor Alan Mellors of the Department of Chemistry and Biochemistry of the University of Guelph for donating the Sigma Lipid Control sera used in this study.

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Acta Medica Hungarica, Vol. 50/1—2, pp. 83—92 (1994)

THERAPY

MYASTHENIA GRAVIS: EFFECT OF IMMUNOACTIVE THERAPIES

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(Received: February 22, 1994)

New immunoactive therapies, plasmapheresis, intravenous steroid pulse infusion and intravenous immunoglobulins were examined for efficacy on the basis of large casuistics of myasthenia gravis. The best results were achieved with combination of these procedures. Indications of the new methods: (i) respiratory crisis of any character (myasthenic, cholinergic or mixed oscillating crisis); (ii) the patients' preparation for thymectomy; (iii) post-thymectomy therapy aimed at improving the patients condition, at avoiding relapses, at shortening the time of steroid therapy and at repressing cholinergic drug therapy; (iv) patients of old age in crisis-prone state. — The new methods, together with thymectomy, steroid therapy and immunosuppression, represent a very efficient and promising new way toward modern therapy of myasthenia gravis.

Keywords: Myasthenia, immunoglobulin, plasmapheresis, pulsus steroid, thymectomy

Introduction

Myasthenia gravis (MG), a disease characterized by disorder of the transmission of neuromuscular stimuli, has long been known as a clinical entity /31/. The disease has gained special importance in the last three decades when MG became the first neurological disease recognized and proved to be of autoimmune character /15—17/. There is another important fact: MG often associates with other known or suspected immune or autoimmune dis-

Abbreviations: AChR: acetylcholine receptor, MG: myasthenia gravis, PEX: plasma exchange, PS: pulsus steroid infusion, IVIG: intravenous immunoglobulin, DSS: Disability Status Scale

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0236-5286/94/$ 4.00

eases; such an association reached 20% in the great casuistics published by one of us /24/, and this fact in itself supports the theory of immune or autoimmune pathogenesis of MG from the clinical point of view. In spite of the fact that quite a few myasthenia syndromes or diseases similar to MG have been described in the last decades /28/, MG itself became a valuable and heterogeneous arena of immunological research, and especially that of therapeutical experiments. It can be considered a model disease in many relations of the question. So the related, mainly therapeutical experiments are of pilot character and may be valid in other research areas, too.

The casuistics of ours -- registered and followed up for 43 years — is one of the greatest MG casuistics in the literature, elaborated in detail concerning all the possible points of view. It is also one of the greatest casuistics for thymectomies and thymomectomies /13, 24, 27, 30/. Extirpation of the thymus gland or of thymoma, the possible total or maximal thymectomy, can be considered the most useful and most valuable procedure in the therapy of MG. Steroid therapy in numerous forms and protocols also has long been used in MG therapy, as has been cytostatic immunosuppression in chronic cases and in relapsing forms of the disease, too /24/. The views mentioned above have opened a way to such newer therapeutic possibilities which essentially improved the results of MG therapy, especially together or in combination with thymectomy. So in the last one and half decades plasmapheresis, plasmaexchange, PEX /4, 10, 14/, later high-dose intravenous methylprednisolone pulse infusion (PS) therapy /2, 25/, still more recently intravenous immunoglobulin (IVIG) therapy /5, 9/ became very important procedures.

Our Department of Neurology can be considered the centre for myasthenic cases in this country; it is used also by foreign patients, concerning both diagnosis and therapy. All the above-listed modern immunoactive therapeutic procedures and their combinations have been applied on a great number of patients (sometimes also with pilot character); it seems to be worth while to give a summary of these modern single and combined therapies, and of the results of their use. We mention here that there are theoretically other modern therapeutical approaches: e.g. antigen manipulation (in form, timespan and efficacy); change of the immune system prior to antigen effect; creation of B cells producing antibodies; suppression of immune reaction specific for AChRs; change of interaction between T and B cells, finally, introduction of antiidiotypic or anticlonotypic reactions, i.e. targeting the antigen-specific T cells. All these theoretical possibilities

are still in an experimental phase; they certainly need a rather long period until one or more of them will be applied in humans /1, 3, 6, 7/. This is a further reason for making available the results of our modern, well-known, proven and applicable therapeutical procedure which are based on our great casuistics.

Casuistics and Methods

Diagnosis, observation, therapy and follow-up of the patients have been based on 43-year experiences of one of us. Meanwhile the view of the disease changed considerably and the term of myasthenia syndrome has been developed; the range of diagnostic procedures widened and so did that of the therapeutical possibilities (except for thymectomy and cholinergic drug therapy).

We surveyed 1596 cases of MG, out of whom 730 passed thymectomy or thymomectomy /13, 24, 27, 30/. The age range of the patients was very wide: 7y - 91y, the majority being 35y - 60y, ($\bar{x}$ = 49.9y, σ = 6.29, σ^2 = 39.6). The age of patients, however, has hardly any influence on the indication of the therapy. Similarly, the sex ratio has no importance (female—male: 2.7:2); it tended to be equalized over the age of 55 years.

The greatest experience — a few hundred patients, more than five hundred treatment — was gained with PEX, which was introduced 6 years after the first experiments in the literature and has been used by us ever since. — PS therapy, i.e. 500-2000 mg methylprednisolone every second or third day, was performed in 56 patients, on 2-5 occasions in each case. — The very expensive IVIG therapy was applied per se only in a few cases, for this method of treatment used alone is of little value, though it is extremely valuable in combination with other active therapeutic procedures.

The therapeutical protocol is shown in a figure (Fig. 1) to make the complicated, sometimes sophisticated forms of these therapeutical combinations easier to understand. Maintenance therapy has frequently been supplemented with long-term azathioprine (Imuran) treatment (100-150 mg daily or on alternate days), less frequently with 100-150 mg levamisole (Decaris) weekly once and, very seldom, with inosiplex (Isoprinosine) or Cyclosporin-A (Sandimmun) therapy.

Although the immunotherapies recognized and introduced recently are of great importance, thymectomy keeps on having its central position in the therapeutic plan of MG, a position that was occupied by this intervention 50 years ago. All the other therapeutical procedures serve the preparation of the patient for surgery, or can be considered supplementary therapies after surgery if necessary; they are applied sometimes repeatedly, or as a maintenance therapy. Hence, all other therapies must be considered in their logical relation to thymectomy or thymomectomy. This complicated therapeutic strategy still pointing in one direction, sometimes even with feed back mechanism, is shown in Fig. 2. The importance of individual decision ought to be stressed in all these patterns, including, of course, the necessary actual changes, too. In spite of the complexity of the therapies listed

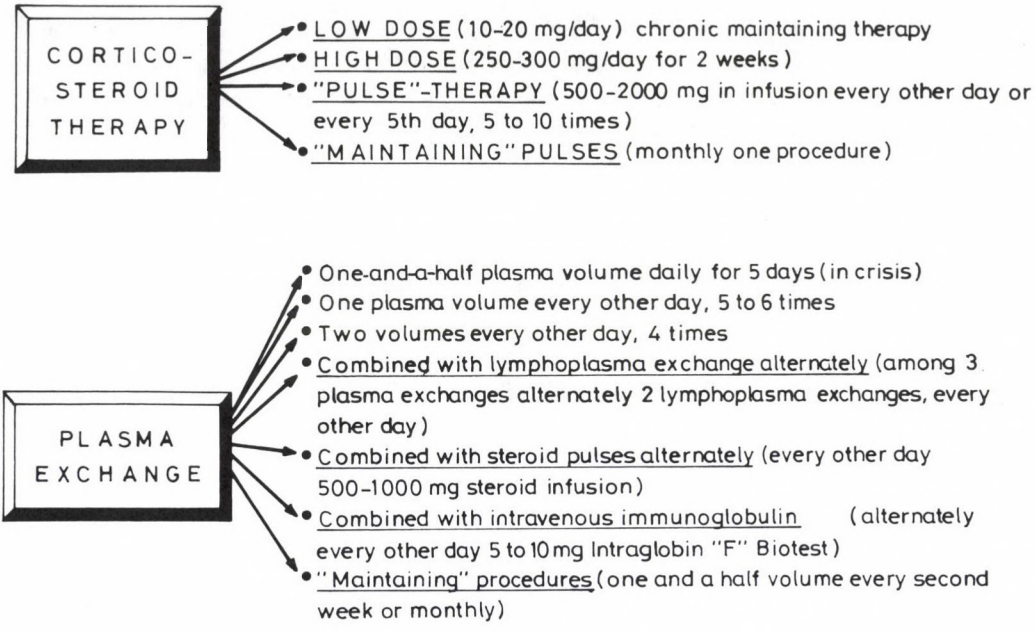

Fig. 1. Therapeutic protocols in myasthenia gravis

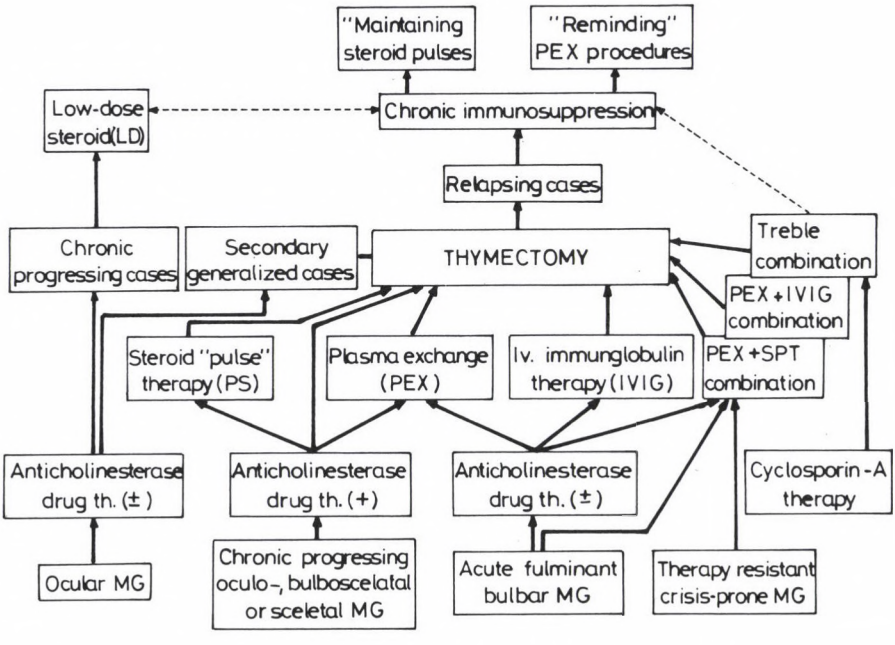

Fig. 2. Strategy for therapy for myasthenia gravis

above, there is no risk of incompatibility when therapy is changed or a new combination is introduced.

Treatment with PEX just as in our previous studies — was performed by a running operative, automatic cell-separator of Dideco-Vivacell type, in general on alternate days or on every third day (in maintenance therapy monthly or bimonthly). The pattern of previous investigations and that of continuous control were described elsewhere /10, 26/, and this pattern has been valid for combined therapies, too. No complications occurred in this great therapeutical series, and considerable side-effects (such as collapse or skin-phenomena) were hardly met. This may be attributed to the careful previous investigations and their correct evaluation. In evaluation, follow-up, and comparison of the patients' clinical status the DSS point system /22, 24/ was applied, which has been used recently by Kornfeld et al. /11/ during the follow-up of great apheresis casuistics.

A considerable part of the patients were admitted in respiratory crisis or in a crisis-prone state. These were treated according to the complicated but well-elaborated rules of intensive care, including utilization of "synaptic resting process" and all the other ways that can be used in this important therapeutic strategy /18—21, 23, 25/. Patients in severe conditions needed constant and rational psychotherapy, i.e. conversation during regularly returning visits, followed by appropriate care and advices /29/. A positive and optimistic spirit of the patient and the solution of his depressive state seem to be important for the function of the immune system, too.

Results

The fields of indication of modern immunosuppressive therapies reflect besides their growing benefit, the increase in therapeutical possibilities. Preparation of patients for surgery has become safer and thymectomy has become possible also in formerly inoperable cases. Thymectomy must be a planned operation which should be performed at the best time, after correct preparation of the patients, possibly ensuring their compliance and consent. Hence, thymectomy is never an emergency operation. Some urgent operations have become possible due to the improvement of the patients' condition and the shortening of the preparation period. These therapies are especially important, for the respiratory crisis or crisis-prone state rapidly ceases and thus the patient can soon be operated. It should not be forgotten that the myasthenic or cholinergic forms of respiratory crisis, as well as the alternating, oscillating manifestations of the crisis present a very difficult problem for intensive therapy /20, 21, 23, 25/. The therapies listed above proved to be essential not only for the quick resolution of crises, but also for the avoidance of rather frequent complications of crises. Results of PEX + IVIG, i.e. PEX + PS treatment, furthermore, that of treble combination for patients suffering from crisis or crisis-prone state, are shown in Fig. 3.

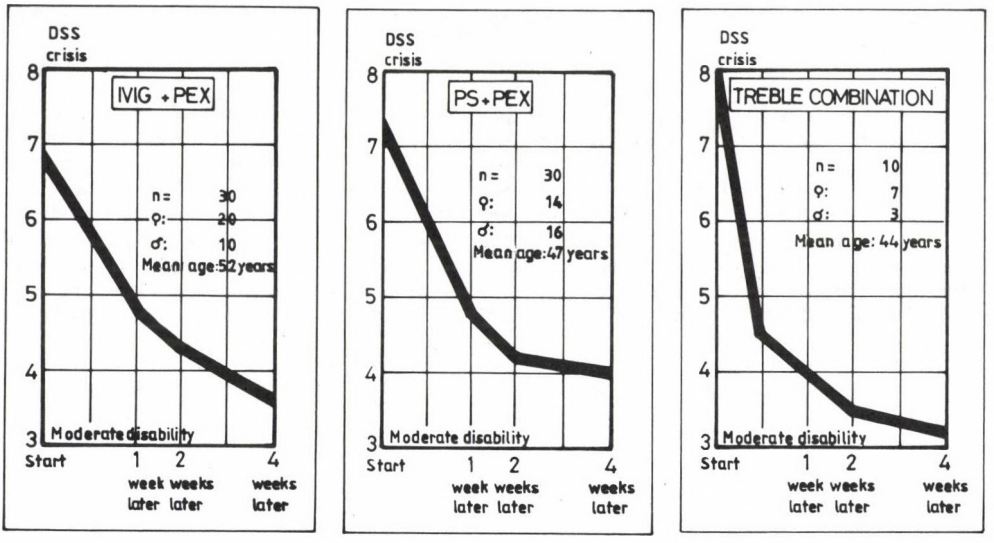

Fig. 3. Effect of combined therapies in myasthenia gravis

The results of even total or maximal thymectomy may not be satisfactory and patients may require further active treatment. The combined therapies have also had an important role in the post-thymectomy therapeutic plan, either in one series or as maintenance therapy.

Recently, one encounter in a growing number of old patients, i.e. cases in which thymic surgery is excluded, except in cases of verified thymoma. Formerly, these patients were only treated by X-ray irradiation of the thymic area in addition to drug therapy. By now the therapeutical methods in these cases have become richer. Consequently PEX treatment has been performed in old patients without any trouble. Furthermore, PS therapy has been well tolerated by the old patients, too, and this invervention has become more efficient than long-term steroid therapy, whose side-effects, including osteoporosis, are considerable. Combined therapy has been found useful in cholinergic drug resistance, moreover, in cases when resistance and intolerance occurred together. PEX therapy may be applied cautiously in pregnant myasthenic patients, too, especially if lack of immunoglobulins can be eliminated by IVIG therapy. Substitution of immunoglobulins is important in elderly patients, too. — Modern immune therapies can be applied in cases associated with other immune disorders. We have gained favourable experiences in MG + multiple sclerosis, MG + polymyositis and in the Landry—Guillain—Barrè—Strohl syndrome.

Discussion

The beneficial effect of PEX in general lasts about one year. This period can be prolonged by half a year, or one year by combination with other interventions. It may be stated that no such important and efficient change has taken place in the therapy of MG since the first application of Neostigmine and development of thymectomy as the introduction of immune therapies, especially in favourable combinations. Furthermore, there is hardly any neurological disorder or syndrome in the therapy of which such an advance would have happened as in MG.

Introduction of PEX therapy in the neurological practice was an event of considerable importance. Sorry, its application is limited by its very high expense. Kornfeld et al. /11/ summarize 10-year experiences in apheretic therapy carried out in an apheretic centre in a great number of cases. Majority of their patients were suffering from neurological diseases, MG or Landry--Guillain—Barre--Strohl syndrome. The authors mention the same considerations in MG practice as we did in our previous studies /10, 26/, and in the present one. The indications are: respiratory crises, grave exacerbations resistant to drug therapy, preparation for surgery, especially of patients suffering from respiratory deficiency, too, post-thymectomy treatment, if cholinergic drug therapy and long-term azathioprine immunosuppression is insufficient, and, finally, avoidening or shortening of a long-term steroid treatment. In pure ocular MG, PEX therapy was not applied in the mentioned casuistics either. Comparing their results with our previous ones /11/, they stress that there was no strict correlation between the duration of MG and the success of the therapy. Patients with AChR antibodies, on the other hand, respond to PEX therapy better than seronegative patients. We failed to find any correlation between the response of the patients to PEX and their antibody level. It can be considered that one of the great advantages of these complex therapies is that the therapeutical response of also seronegative cases is good, although immunoglobulins in these instances may be bound to a target-organ (muscle) and the clinical improvement can be ascribed only to the elimination of circulating immune-complexes.

PS therapy, an intervention used far less frequently than PEX, was found successful when used alone /25/ and still better in combination with PEX. In addition to its efficiency, it has a further important advantage: it can partly or completely substitute the long-term steroid cure, thus decrease or even eliminate its side-effects. We were surprised to learn how

well elderly patients tolerated PS therapy. The effect with combination with PEX was almost dramatic in very serious and hardly treatable patients, too, even in crisis-prone or resistant cases.

IVIG therapy in itself was poorly efficient. Considerable effect was observed, however, in combination with either PEX or PS. A treble combination, however, was performed only in very few cases, just as ultimum refugium. Advantage of IVIG treatment can be looked for in quick substitution of the immunoglobulins lost in consequence of PEX therapy. This may be important in old patients, in cases with weakened organism, in patients with intercurrent disease or in pregnancy.

In spite of the fact that previous literary data /12, 24/ unequivocally proved that in the cultured areas of the world MG did not depend on geographical, social or nutritional factors, long-term research shows a slow increase in MG incidence. This fact should be attributed not only to the more improving diagnostic possibilities of MG, but also to an apparent density of the disease both in childhood and in older age. This includes, of course, an increased number of childhood myasthenic syndromes /8/, as well as of the associated and provoked myasthenia syndromes /28/. The ratio of original, acquired MG -- as an entity — has shown a modest increase.

We consider on the basis of the above-mentioned facts that a correct therapeutic plan should be elaborated for the very efficient, modern immunotherapies applicable in the acquired forms of MG. For this purpose, their indications should be considered primarily. So, respiratory crisis of any kind, especially oscillating crisis characterized by myasthenic and cholinergic symptoms (existing sometimes simultaneously), has been considered prime indication for combined therapy. These methods have been efficient, even life-saving. Thymectomy or creation of its conditions often need careful preparation. By routine use of these modern immunoactive therapies, operation became feasable even in previously inoperable cases. Accordingly, the same is valid also for the post-thymectomy period when the result of surgery is poor, when drug therapy is not satisfactory on account of resistance, intolerance or both, or when the duration of the long-term steroid therapy ought to be shortened. These therapies may be indicated in associated diseases, in childhood, in old age, and sometimes in pregnancy, too. Double combination in general should be planned in each case individually, on the basis of the duration, clinical form and therapeutical responsiveness of the disease. Omission of long-term steroid therapy is a special advantage. Complications practically do not occur if patients were prepared cor-

rectly. In such cases, furthermore, side-effects are less pronounced, occur very rarely, they do not risk the patient.

Hence, the therapeutic inventory of MG has been essentially enriched by the immunoreactive therapies, which — together with thymectomy, steroid and cytostatic treatment — significantly improved the life-quality of our patients, furthermore, they may open gate for new directions of research and promise further good results in the near future.

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Acta Medica Hungarica, Vol. 50/1—2, pp. 93—98 (1994)

POLYPHASIC AND PROTRACTED PATTERNS OF HEPATITIS A INFECTION: A RETROSPECTIVE STUDY

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(Received: June 14, 1994)

The present study was performed in order to evaluate the frequency and clinical features of polyphasic and protracted forms of HA infection. Out of 297 adult HA patients admitted to our hospital, 12.5% and 8.4% were polyphasic and protracted, respectively, in clinical course. 21.6% of the polyphasic patients had more than one relapses. The rates of symptomatic relapse during the follow-up of the polyphasic and protracted HA infections were 51% and 56%, respectively, One % of all HA patients presented a cholestatic pattern. Three % of all the patients had to be readmitted for hospital treatment. The outcome of disease was benign in all HA patients, though, complete recovery sometimes needed six months.

Keywords: Hepatitis A infection, polyphasic, protracted, cholestatic patterns

Introduction

Recently the incidence of hepatitis A had decreased in Hungary /7, 13/, indicating an increase in susceptible population. HA is usually a mild and self-limited disease, but it may take a protracted, or polyphasic course /1—6, 11/, especially in adults. However, it remains generally benign; it resolves in six months.

In the recent years a few reports of sporadic cases of persistently abnormal liver function with IgM anti-HAV, and chronic histological changes in HA patients have been documented /8—10/.

Abbreviations: HA: hepatitis, HAV: hepatitis A virus, anti-HAV IgM: hepatitis A virus antibody class M, HBV: hepatitis B virus, HBsAg: hepatitis B surface antigen, HBc IgM: hepatitis B core antibody class M, HCV: hepatitis C virus, anti-HCV IgG: hepatitis C virus antibody class G, CMV: cytomegalovirus, EBV: Epstein—Barr virus

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0236-5286/94/$ 4.00

The prevalence and the clinical features of polyphasic and protracted patterns in HA patients are evaluated retrospectively.

Material and Methods

Patients: 297 adult HA patients were consecutively admitted to the 3rd department of Internal Medicine of the Szent László Hospital in Budapest, from January 1988 to September 1993.

Demographic data and disease characteristics are shown in Tables I, II and III. Diagnosis was based on clinical features, laboratory findings, and positive IgM anti-HAV tests. All the patients with atypical course were negative to HBV, CMV and EBV. HCV IgG test was introduced for exclusion of HCV, as late as in January 1990. There were no intravenous drug abusers, homosexuals, alcoholics among the patients, nor people who had undergone a recent blood transfusion or immunosuppression therapy; extrahepatic obstruction and drug-induced icterus were also excluded. Liver function tests (ASAT, ALAT, ALP, GGT) were performed weekly during the acute phase of disease and monthly (3-5 weeks) during the follow-up period. The standard methods were used. Assay for anti-HAV IgM, HBsAg, HBc IgM were carried out using Organon Teknika Microelisa, Roche Cobas-core automated ELISA. Tests for HCV used Organon Teknika Microelisa (first generation), Ortho, Abbott (second generation). CMV was investigated by Organon Teknika Microelisa, Biowhittaker EIA. Tests for EBV were performed by immunofluorescence methods. All sera were stored at -20 ^{o}C until tested. We regarded the clinical course typical when acute icteric period resolved within 1-4 weeks, followed by permanent normalization of ASAT, ALAT by the 12th week. The term "polyphasic course" was used to HA patients who had a 2-10 times increase in ASAT, ALAT with or without elevation in serum bilirubin level after the early convalescence period. The term "protracted course" was used in cases when the acute phase was severe and slow resolution in liver function values lasted more than 12 weeks. Cholestatic pattern was applied to patients who had a prolonged severe jaundice with pruritus accompanied by highly elevated ALP and a bad general state.

Results

The 62 HA patients who showed an atypical course represented 20.9% of the admitted HA patients; of these 37 (12.5%) showed a polyphasic, and 25 (8.4%) a protracted clinical pattern.

Characteristics of polyphasic HA cases are illustrated in Tables I and II. Eight of these patients (21.6%) presented more than one relapse.

The average interval between the early convalescence and the first relapse was five weeks, and it was four weeks between the relapses.

We noted that during the relapses only 51% of polyphasic HA patients presented symptoms such as anorexia, and/or fatigue, epigastric discomfort with or without jaundice. Seven polyphasic HA patients were readmitted to the department for bed-rest and symptomatic treatment. Tables I, III give the features and liver function values of 25 protracted HA patients. Three

Table I

Characteristics of typical and atypical patterns of HA infection

	Typical course	Polyphasic course	Protracted course
No. of patients	235	37	25
Men/Women ratio	0.98	1.1	2.1
Age (years), mean (range)	27.8 (15—65)	22.8 (14—78)	27.9 (14—48)
Length of acute phase mean (range), days	16 (4—45)	17 (13—45)	29 (17—95)
Total length of illness mean (range), days	46 (27—60)	117 (67—186)	106 (79—159)
No. of readmitted patients	0	7	2
No. of patients who presented symptoms during follow-up	0	19	14

Table II

Laboratory findings in polyphasic cases

	Acute phase	Early convalescence phase	1st relapse	2nd relapse
ASAT* (U/1)				
mean (range)	670 (99—2850)	88 (16—310)	267 (73—310)	320 (81—410)
ALAT** (U/1)				
mean (range)	1232 (185—3790)	179 (54—298)	507 (137—1380)	479 (188—1090)
Serum bilirubin*** (mmol/1)				
mean (range)	122 (17.1—147)	33 (17.1—78)	24.3 (17.1—38)	19 (17.1—25)

*normal range < 37; **normal range < 40; ***normal range < 17.1

of them (1% of the total HA patients) showed a cholestatic pattern, total serum bilirubin ranged between 291 and 660 mmol/1. The clinical recovery and normalization of liver function tests of the HA patients with protracted course appeared at 3, 4, 5 and 6 months, 52%, 36%, 4% and 8%, respectively. Forteen of the protracted cases (56%) were symptomatic during the follow-up,

Table III

Laboratory findings in protracted HA cases

	Acute phase	Early convalescence phase	1st follow-up
ASAT* (U/l)			
mean (range)	821 (118—3400)	110 (43—1250)	97 (58—301)
ALAT** (U/l)			
mean (range)	1257 (229—4600)	265 (75—1436)	190 (82—570)
Serum bilirubin*** (mmol/l)			
mean (range)	159 (42—660)	46 (17.1—294)	27 (17.1—126)

*normal range < 37; **normal range < 40; ***normal range < 17.1

and two of them needed readmission to the department with symptoms similar to that seen during the acute-phase disease.

In general, the overall rate of rehospitalization because of relapse was 3%.

Discussion

Many hypotheses have been suggested to explain the pathogenesis of relapsing course of HA infection, such as a supposed defect in cell-mediated immune mechanism, mutation in HAV, superimposed infection with other viral agents. Sjogren et al. /12/ isolated HAV from the stool of HA patients during the relapse by using immune electron microscopy and molecular hybridization; accordingly, HAV itself seems to play a causative role in the aetiology of relapsing hepatitis A. Villari et al. /15/ found histological changes of the liver during the relapse phase of HA infection are similar to other acute hepatitis due to viral agents.

Our findings have shown that polyphasic course occurs more often than it was previously suggested /2, 6, 11, 14/, we did not find any difference in mean age, sex, and mean duration of the first attack between HA in the

polyphasic HA group and the typical HA patients. Mean values of ASAT/ALAT during the relapses were three times higher than the values of the early convalescence phase, in contrast with serum bilirubin which was lower during the relapse than in the early convalescence phase.

Age, underlying disease, and other predisposing factors have been observed in protracted HA infection. We did not find any prevalence of predisposing factors in our patients with protracted course, except that these patients were seriously ill at the onset of the disease. The only laboratory sign of protracted disease was a low prothrombin rate (0.40 or less), which may be a consequence of massive liver cell necrosis.

The clinical features of the acute phase in polyphasic HA infection were similar to that of typical HA infection without any sign that may be helpful in predicting relapse.

Generally, all atypical HA infection were benign in outcome, and the disease resolved entirely in complete recovery within six months.

In conclusion, HA infection may not be a short-course disease; it may take a severe, polyphasic, and slow-resolution course, therefore, it merits a careful, and sufficiently long follow-up.

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Acta Medica Hungarica, Vol. 50/1–2, pp. 99–107 (1994)

LABORATORY

THE USE OF URINE PROTEIN 1 AS AN INDICATOR OF RENAL TUBULAR FUNCTION IN TYPE I (INSULIN-DEPENDENT) DIABETES

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(Received: March 25, 1993)

One hundred male insulin-dependent diabetic patients, aged 16 to 85 (mean 51.9) years, with albumin excretion ranging from normal to gross excess were examined for glomerular and tubular functional alterations by estimating urinary levels of albumin and indicator proteins of tubular damage. Urine protein 1 (UP1), a newly-discovered low-molecular weight alpha-2 glycomicroglobulin, together with α_1-microglobulin was used to assess tubular function. 19% of the patients showed increased albumin excretion with normal levels of tubular proteins (glomerular proteinuria), 11% excreted only tubular proteins in excess (tubular proteinuria), while 40% had a mixed pattern of both increased albumin and tubular proteins (glomerulotubular or mixed proteinuria). 30% had normal albumin and tubular protein excretion in urine. UP1 was found to be a more sensitive indicator of tubular abnormality than α_1-microglobulin. It is concluded that, although glomerular changes may be responsible for the proteinuria seen in most diabetics (mixed proteinuria), in a small but significant proportion of diabetics, tubular functional alteration may occur before, or in the absence of, glomerular dysfunction, and may warn of subclinical tubular abnormality. This finding may have a direct bearing on the development and course of progression of diabetic nephropathy, and may question the reliability of the present prognostic interpretation of microalbuminuria.

Keywords: Urine protein 1, proteinuria, kidney disease, albuminuria, tubular dysfunction, diabetes mellitus

Introduction

Diabetic nephropathy has been identified as a major cause of renal failure in patients beginning therapy for end-stage renal disease. It has been estimated that about 30% of insulin-dependent diabetic mellitus subjects will develop proteinuria and progressive renal failure on the average

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of 16 to 20 years after onset of the disease /10/. This is because when diabetic renal disease is well established, as evidenced by clinical proteinuria or albuminuria, attempts to modify the relentless progression of the disease have often failed /10/. The dominant role of diabetic nephropathy as a cause of death in late diabetes stresses the importance of studying changes in the renal function of diabetics.

Reports characterizing a much earlier stage of diabetic renal disease, called microalbuminuria /20/, have called attention to the early identification and therapeutic intervention, with a view to delaying the rate of progression to end-stage renal disease. The early renal changes in diabetics can be glomerular or tubular. However, since diabetic kidney disease is usually clinically characterized by an increased urinary excretion of albumin (a generally accepted marker of glomerular malfunction), it has often been argued that diabetic nephropathy is exclusively a glomerulopathy /17, 21/. In the last decade, a growing body of evidence has been advanced which appear to call for more studies on tubular involvement in diabetes. These studies indicate the presence of tubular proteinuria before clinical nephropathy develops /11, 19/.

The present study reports the estimation of tubular involvement in diabetic patients by assaying Urine Protein 1 (UP1), which is an α_2 glycomicroglobulin (Mr 21000) that was isolated from human patients with renal tubular dysfunction (Dakopatts, Denmark). It has been partially purified, sequenced and characterized /8, 12/. The origin and function of UP1 has not been fully ascertained but data suggest that it might be useful as a new marker of impaired tubular reabsorption /1, 2, 7/. Excretion of α_1-Microglobulin (Mr 33000) which has been widely used in evaluating tubular function, was also examined.

Urinary albumin excretion was used to monitor glomerular function. Diabetic metabolic control was assessed by the levels of red cell glycosylated haemoglobin (HbA_{1c}).

Patients and Methods

Patients

One hundred male insulin-dependent (type I) diabetes mellitus patients, aged between 16 and 85 (mean 51.9) years, with albumin excretion ranging from normal to overt albuminuria, were included in our studies. The patients supplied untimed early morning urine and overnight fasting blood samples. From a control group consisting of 50 male healthy volunteers, aged

20 to 50 years, early morning urine samples were obtained. None of the patients and control subjects showed evidence of urinary tract infection. All urine samples were stored at -20 °C until analyzed.

Assays

Red cell glycosylated haemoglobin (HbA_{1c}) was measured by electroendo-osmosis using the Glytrac™ System (Ciba-Corning Ltd., Essex, U.K.). The between batch coefficient of variation of the method was 4% at HbA_{1c} concentration of 12%. Urinary albumin was measured by immunoturbidimetry on a centrifugal analyser (Roche Diagnostics, Welwyn Garden City, Herts, U.K.). This method had an interassay variation of 4.6% at albumin concentration of 15 mg/L, and a recovery of 98.7%. For urine creatinine assay Jaffe's picric acid reaction on a centrifugal analyser was used. α_1 microglobulin (α_1M) and UP1 were measured by radial immunodiffusion /14/ and ELISA /22/, respectively. Statistical evaluation was performed using Student's t-test.

Results

Of the 100 patients studied 12 had gross proteinuria with urinary albumin >230 mg/L. All urinary proteins measured were expressed as ratio of protein to creatinine, as recommended by Barrat /5/ and Marshall and Albert /15/: albumin to creatinine (ACR), UP1 to creatinine (UCR) and α_1M to creatinine (MCR). The upper limits of normal (mean $\pm$ 2 SD) were for ACR 1.5 mg/mmol, for UCR 30 µg/mmol, and for MCR 1.2 mg/mmol creatinine, above these levels exretion was considered abnormal. Patients with ACR > 2.5 mg/mmol creatinine and a negative reaction to Albustix were classified as having microalbuminuria.

The diabetic population had red cell HbA_{1c} levels of 9.9 $\pm$ 2.2% (range 6.7 to 18.8%), and there was a significant difference ($p < 0.01$) between the

Table I

Classification of renal function of diabetic subjects*

Urinary protein pattern	Type of proteinuria	Renal function	n
Normal albumin, UP1 and α_1M	Normal	Normal	30
Increased albumin, normal UP1 and α_1M	Glomelurar	Glomerular dysfunction	19
Increased UP1 and α_1M, normal albumin	Tubular	Tubular dysfunction	11
Increased albumin, UP1 and α_1M	Glomerular and tubular	Glomerular and tubular dysfunction	40

*UP1 = Urine protein 1; α_1M = α_1-Microglobulin

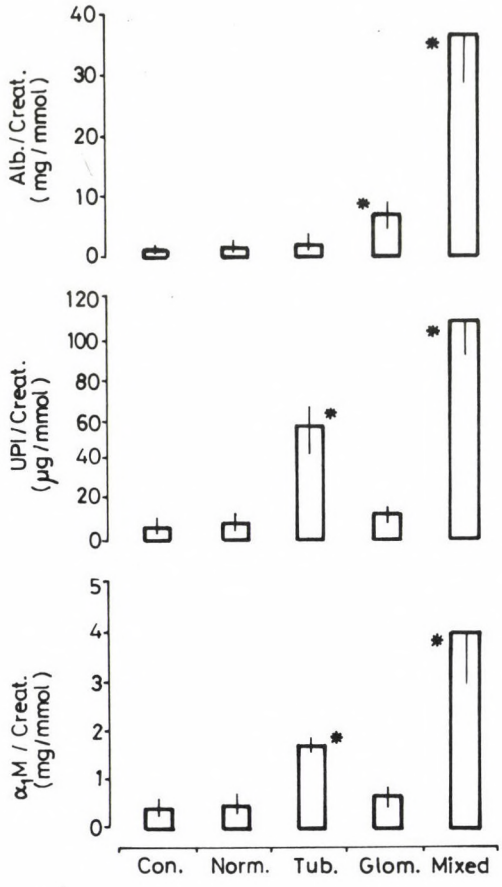

Fig. 1. Urinary output of albumin, α_1-microglobulin (α_1M) and Urine Protein 1 (UP1) relative to creatinine in control subjects and diabetic patients. Con. = control or healthy subjects (normal proteinuria, n = 50); Norm. = diabetic subjects with normal proteinuria (n = 30); Tub. = diabetics with tubular proteinuria (tubular dysfunction, n = 11); Glom. = diabetics with glomerular proteinuira (glomerular dysfunction, n = 19); Mixed = diabetics with glomerular and tubular proteinuria (glomerulotubular dysfunction, n = 40). Types of proteinuria are as defined in Table I. Mean ± SEM are indicated. *Difference vs. control: $p < 0.001$

levels in normal subjects and diabetics. The correlation between HbA_{1c} and all the low-molecular weight proteins was however poor.

The relationships between the albumin and the globulin proteins was estimated. Above ACR of 2.5 mg/mmol, there was a significant correlation between ACR and UCR ($r = 0.71$, $p < 0.001$, $n = 62$) and between ACR and MCR ($r = 0.88$, $P < 0.001$), $n = 62$). This contrasted with the poor correlation between ACR and UCR ($r = 0.09$, $p < 0.6$, $n = 38$) or ACR and MCR ($r = 0.29$, $p < 0.4$,

n = 38) for ACR below 2.5 mg/mmol. A significant correlation was however shown between the urinary globulin proteins ($r = 0.91$, $p < 0.001$, $n = 100$).

The glomerular and tubular functions of the patients were characterized by the ratios ACR, UCR and MCR. Patients with ACR >2.5 mg/mmol were considered to have glomerular dysfunction, while those with increases in both MCR and UCR (> 1.2 mg/mmol and 30 µg/mmol, respectively) were considered to have tubular impairment. Based on these urinary proteins, the renal function of the diabetic patients have been classified as shown in Table I.

There was a good agreement (91%) between MCR and UCR. However, discordant globulin values were noticed in 9 of the patients. Urine samples from these patients were further examined using retinol-binding protein (RBP), which is a more sensitive plasma protein for tubular function. RBP was assayed by ELISA /3/. Using RBP, there was a 8/9 agreement with the trend as shown by UP1 in contrast with the 2/9 agreement found with α_1M. UP1 may be more sensitive or reliable in detecting renal changes than α_1M.

All the 12 patients with Albustix-positive urine showed glomerular and tubular (or mixed type) renal dysfunction. Data for the urinary protein excretion (mean ± SEM) are shown in Fig. 1.

Discussion

We used urinary albumin, UP1 and α_1M levels to assess glomerular and tubular involvement in a cross-section of 100 diabetic patients. There was a good agreement between the newly discovered protein UP1 and α_1M ($r = 0.91$), indicating that UP1 is a good monitor of renal function in this group of patients. However, when compared with RBP, the globulin "gold standard" for tubular function /6, 16/, UP1 appeared to be more sensitive than α_1M in detecting tubular functional changes in diabetics. This is evidenced from the good correlation between UP1 and RBP in the nine samples with discordant UP1 and α_1M values. This sensitivity of UP1 over α_1M in monitoring renal function agrees with earlier reports /2, 4/.

Eleven out of the 41 normoalbuminurics (albumin 2.5 mg/mmol) had increased urinary UP1 and α_1M excretion (tubular proteinuria). This is in agreement with observations by Rowe et al. /19/, but at variance with reports of others /16, 20/, who used β_2-microglobulin as indicator protein for renal function. This discrepancy may be due to the greater instability of β_2-microglobulin vs. α_1M and UP1 /1, 14/, and the use of 24-h urine without

alkalinization /20/, which provided a longer period for the breakdown of β_2-microglobulin in that study. In addition, increased acidity of urine during periods of poor control of diabetes might be expected to increase the liability of β_2-microglobulin and thus conceal obvious tubular abnormity.

There was a weak correlation between HbA_{1c} and α_1M or UP1, indicating the lack of relationship between renal impairment and the degree of glycaemic control as shown by glycosylated haemoglobin concentration. This may mean that, although metabolic derangements may have initiated the nephropathic events, the processes determining renal deterioration in the proteinuric stage are independent of glycaemia. Glomerular changes that occur during poor metabolic control, such as functional microangiopathy with increased filtration pressure and/or increased porosity of the microvasculature /19/, are not likely to affect urinary excretion of small plasma proteins since their glomerular permeability is usually high /13/. Also the generally fairly good metabolic control of the diabetics in this study (HbA_{1c} = 9.9 $\pm$ 2.2%) may explain the fact that there was a very poor correlation between urinary albumin and red cell HbA_{1c}.

Urinary excretion of UP1 and α_1M was frequently increased in the group of patients with increased albumin excretion. This may be due to two possibilities. First, it may indicate progressive renal damage advancing perhaps through glomerular damage to involve the renal tubules. This would be consistent with the high urinary levels of small plasma proteins observed in all patients with gross proteinuria. Second, increased albumin in the ultrafiltrate due to damaged glomeruli may saturate and inhibit the reabsorptive mechanisms of otherwise normal proximal tubules /13, 18/, and precipitate increased tubular proteins and albuminuria.

This study has shown that in 11% of diabetic patients, tubular proteinuria (indicated by increased excretion of α_1M and UP1) and hence tubular dysfunction, may, besides occurring early before the positive conventional Albustix test, occur independently of glomerular change or microalbuminuria. This finding poses a number of questions. Do the tubular proteins occurring without albuminuria have any prognostic message? Does the presence of tubular proteinuria render prognostic interpretation of microalbuminuria unreliable? Clearly, only long-term prospective studies (using purely tubular, glomerular and mixed type proteinuric diabetics) can provide answers and give empirical evidence for the prognostic value of the estimation of tubular proteins in diabetics. However, the implication of tubular proteinuria on the interpretation of microalbuminuria seems obvious. First, low-

molecular-weight (LMW) proteinuria is an indication of proximal tubular malfunction, which was previously regarded as a relatively late complication of diabetic renal disease /17, 20, 21/. Second, the interpretation of microalbuminuria usually depends on the assumption that tubular reabsorption is unimpaired and therefore microalbuminuria is concluded to be a reflection of a glomerular change. The present result undermines this assumption because it arises from tubular lesion which may impair tubular reabsorption of albumin. This implies that microalbuminuria may be a less specific indicator of glomerular changes in diabetics than it was assumed.

Let us examine further the implications of tubular proteinuria in the prognosis and management of diabetic nephropathy. Consider two groups of patients: the one with normal and the other with dysfunctional proximal tubules; both have normal initial glomerular function. Assume that both subsequently develop structural and functional glomerular alterations. In the group with normal tubular function, albumin reabsorption would be efficient, therefore, ultrastructural and functional glomerular changes may be correctly revealed by albumin excretion. Correct prognostic signals may be read with respect to the renal disease. This is the group where current interpretation of albuminuria is applicable. In the group with pre-existing proximal tubular dysfunction, there would be an inappropriately high albumin excretion in relation to the level of glomerular alteration. This would give wrong prognostic signals of the renal disease. The result would be a poor correlation between morphological changes in the kidney and renal function or the development of renal disease. It has been reported, for example, that whereas 30-40% of all insulin-dependent diabetic patients will develop overt clinical nephropathy, 60-70% of the patients never develop clinical renal disease despite histological evidence of glomerulosclerosis in nearly all patients after only a few years of the disease /9/. Patients with tubular dysfunction may show a different clinical course of renal disease from those without tubular abnormity. This result therefore advises caution in the interpretation of albuminuria in diabetics, especially when the history of tubular function is unknown.

The present study using the senstive LMW protein UP1, has shown the presence of tubular proteinuria in diabetics without microalbuminuria. Although it is presumptious based on the available empirical data to conclude that the evaluation of tubular proteinuria can, at the present state of knowledge, improve the sensitivity and specificity of predicting renal desease in diabetics, the evidence presented here raises questions that

challenge the scientific premise on which both the prognostic signal and the interpretation of microalbuminuria are based. Long-term prospective studies may provide data that would reveal the actual role of tubular proteinuria in the sensitivity and specificity of predicting overt diabetic nephropathy.

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Acta Medica Hungarica, Vol. 50/1—2, pp. 109—115 (1994)

MITOTIC DELAY IN PERIPHERAL BLOOD LYMPHOCYTES AND FIBROBLAST CULTURES OBTAINED FROM A CHILD WITH DOWN'S SYNDROME AND FROM A HEALTHY CHILD

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(Received: September 18, 1994)

Mitotic delay (MD) often occurs in cells of donors exposed in vivo to genotoxic agents. To investigate individual sensitivity with genetic background, author measured the 3-methylcholanthrene (MC)-induced MD in cultured human skin fibroblasts (FBs) and in peripheral blood lymphocytes (PBLs) obtained from a 4-year-old patient with Down's disease. Samples from a 10-year-old healthy subject served as controls. Skin samples were obtained during surgical intervention. The induced MD was calculated from the mitotic index (MI) which was expressed in per cent of the control; at various times up to 18 h after treatment. Cells were treated with 10^{-7}, 10^{-6} and 10^{-5} M MC (with S-9 liver homogenate). At passage 10, the average MI ($\pm$ SE) was $8.32 \pm 0.43\%$, and $7.85 \pm 0.64\%$ for the healthy and for the Down's FBs, respectively; and it was $4.89 \pm 0.59\%$, and $4.92 \pm 0.72\%$ for the healthy and for the Down's PBLs, respectively. MD was characterized as 50% MI of control (MD_{50}). The MD_{50} values were the most expressed when cells were treated with 10^{-5} M MC. No difference was found in MD of healthy and Down's fibroblasts. For Down's lymphocytes, on the other hand, MD was approximately 30% longer than for healthy cells. This result agrees well the reported increased SCE and decreased DNA-repair data obtained in PBL of Down patients.

Keywords: Children, genotoxicology, individual sensitivity, in vitro 3-methylcholanthrene-treatment, risk assessment

Introduction

Mitotic delay (MD), in general represented in reduced proliferation index at the time of standard sample preparation is often observed in peripheral blood lymphocytes (PBLs) of populations exposed to genotoxic agents /8, 20/. Reduced proliferation index /5/ is also often associated with decreased unsheduled DNA synthesis /9, 20/. Individual sensitivity with a genetic background including DNA-repair capacity, influences the obtained

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data; it is a current topic of genotoxicology monitoring. Patients with Down's disease are more sensitive than healthy donors to in vitro exposures when measured with SCE /10/. Lambert et al. /4/ found decreased UV-induced DNA repair, and Agarwal et al. /1/ reported decreased DNA polymerase activity in patients with Down's syndrome. Therefore, investigation of cells obtained from patients with Down's syndrome can help in a better understanding of individual sensitivity to genotoxic exposures.

Survival and MD after X, or γ irradiation was intensely studied in various type of cells from healthy donors /7, 17/ and from patients with ataxia teleangiectasia /7, 12, 16, 17, 21—23/ or with retinoblastoma /22/. There is an evidence of a relationship between MD and DNA-lesions in X-ray irradiated cells /2, 16, 19/; and between MD and DNA repair /25/. However, less attention has been paid to chemically induced MD. No information is available on induced MD in cells obtained from patients with Down's syndrome. The aim of this study was to investigate 3-methylcholanthrene (MC)-induced MD in cultured human skin fibroblasts (FBs) and PBLs. A healthy child, and a patient with Down's disease were the donors.

Materials and Methods

Cell cultures

Cell cultures were set up as described elsewhere /10/. Briefly, primary skin FBs of a 10-year-old healthy child, and a 4-year-old patient with Down's syndrome were cultured under standard conditions in TC-199 medium (Flow) supplemented with 20% fetal calf serum (Flow) on glass coverslips in Petri dishes; cell strains underwent for 20 passages. The skin samples were obtained during surgical intervention. MD studies were performed at passage 10, the passage in which the trisomy of the chromosome 21 was present. Peripheral blood lymphocytes were taken by venipuncture from the same donors, and cultured under standard conditions, for 68 h in RPMI-1640 medium (Flow) supplemented with 20% fetal calf serum (Flow), and 5 µM phytohaemagglutinin-M (Bacto) in sterile glass tubes.

Treatments and determination of MD

The MC (CAS Reg. No.: 56-49-5, Sigma) chosen for treatment was in concentration 10^{-7}, 10^{-6} and 10^{-5} M (in the presence of S-9 liver homogenate). Lymphocyte cultures were treated for 10 min, 50 h after the cultures were set up, then the medium containing MC was washed out, and replaced with fresh medium. FBs were treated 24 h after passage for 10 min, then the medium was changed.

Sample collection was taken at 0 h, 0.5 h, 1 h and 2 h after treatment, then in 2 hours intervals, up to 18 h. The cells were then fixed in situ, in acetic acid:methanol, 3:1 (PBL then dropped onto slides, and air dried); and stained with 5% Giemsa (Fluka). MD was determined according to Scott and Zamptetti-Bosseler /16/. Frequencies of all mitotic phases per 2000

interphases were determined in each sampling time, both for FB and PBL. MIs in the treated samples were calculated as a ratio to MI of the untreated control of the given sampling time, and expressed in % of the control. MD_{50} (i.e., the difference in hours between the corresponding two MI values at 50% of the control) was calculated according to Scott and Zampetti—Bosseler /10/. Significances were determined by the t-test.

Results and Discussion

At passage 10, the average MI (± SE) was 8.32 ± 0.43%, and 7.85 ± 0.64% for the healthy and for the Down's FB, respectively; and it was 4.89 ± 0.59, and 4.92 ± 0.72 for the healthy and for the Down's PBL, respectively. MI of the untreated controls as a function of sampling time is demonstrated in Fig. 1. The difference was significant neither between healthy and Down's lymphocytes, nor between healthy and Down's fibroblasts. As expected, the mean MI for lymphocytes /3/ was significantly lower ($P < 0.01$) than MI for fibroblasts.

Figure 2 shows MI in MC-treated cells as % of the actual untreated control. However, 10^{-7} M MC-treatment resulted in no mitotic delay either in fibroblasts or in lymphocytes. In Fig. 2A results are presented on 10^{-6} M MC-treated FBs. For healthy, and Down's FBs, MD was 12.0 h, and 11.0 h, re-

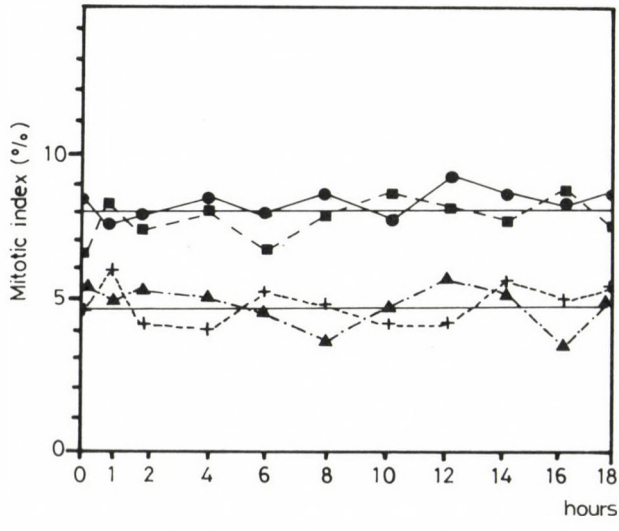

Fig. 1. Mitotic index (%) for the investigated untreated (control) cell cultures. Data represent the actual 100% mitotic index for the investigation of induced miotic delay after 3-methylcholanthrene treatment. —●— Healthy skin fibroblasts; — ■ — Down's skin fibroblasts; - · ▲ · - Healthy blood lymphocytes; - - + - - Down's blood lymphocytes

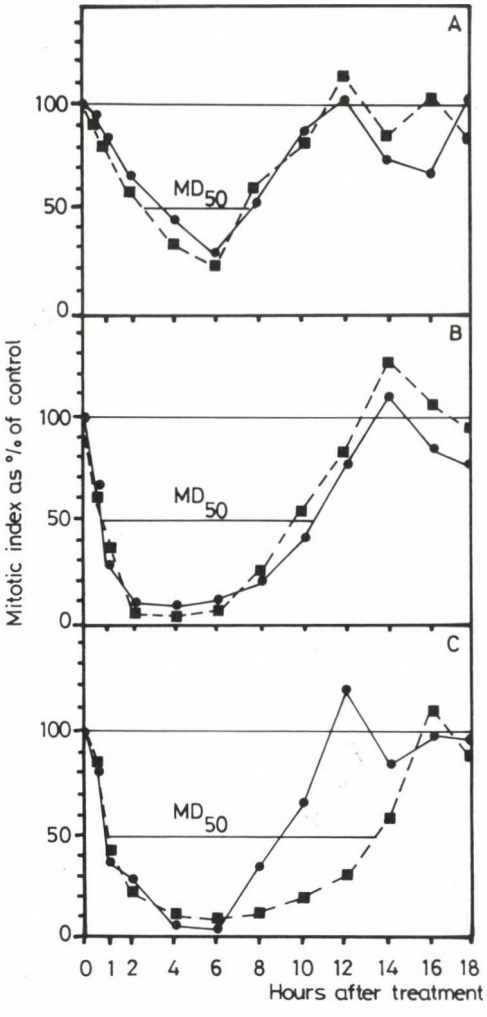

Fig. 2. Demonstration of mitotic delay (expressed as % of the actual untreated control mitotic index) in 3-methylcholanthrene (MC)-treated cell cultures (with S-9 liver homogenate). MD_{50} (indicated as a bar) represents the mitotic delay at 50% mitotic index of the control. A) Mitotic delay in 10^{-6} M MC-treated healthy and Down's fibroblasts; —●— Healthy skin fibroblasts; — ■ — Down's skin fibroblasts. B) Mitotic delay in 10^{-5} M MC-treated healthy and Down's fibroblasts; —●— Healthy skin fibroblasts; — ■ — Down's skin fibroblasts. C) Mitotic delay in 10^{-5} M MC-treated healthy and Down's lymphocytes; —●— Healthy blood lymphocytes; — ■ — Down's blood lymphocytes

spectively; MD_{50} was 4.2 h, and 4.6 h, respectively. The differences were not significant. Mitoses were found in each sampling time. Lymphocytes showed no measurable MD. MI values were over 50% of the control in each sample. In Fig. 2B results are demonstrated on 10^{-5} M MC-treated FBs. MD was

13.0 h and 12.5 h; MD_{50} was 9.8 h and 9.0 h for the control, and for Down's FBs, respectively. The differences were not significant. No mitoses were found between 2 h and 6 h after treatment. An "overshoot" was observed 14 h after treatment, indicating synchronization of cultures /21/. Results obtained in 10^{-5} M MC-treated lymphocytes are presented in Fig. 2C. For treated healthy lymphocytes MD was 11.0 h and MD_{50} was 8.0 h. However, MD was 15.5 h and MD_{50} was 12.2 h in the case of 10^{-5} M MC-treated Down's lymphocytes. The difference in MD_{50} between healthy and Down's lymphocytes is significant 34.4% ($P < 0.01$). No mitoses were found 4-8 h after treatment. The kinetics of the MI curves represent the mitotic phases. In the first 2-4 h after treatment mainly telophases were observed. Prophases appeared 6-8 h after treatment.

The ionizing radiation-induced MD was prolonged in human PBL, He-La, mouse lymphoma, and chinese hamster ovary cell cultures /2, 15, 16, 24, 25/. In FBs from patients with ataxia teleangiectasia, however, X-ray irradiation /7, 21, 22/, and UV-light irradiation reduced MD /12/. The prolongation or reduction of MD is connected with the DNA-repair activity of the cells /11, 13, 18, 23/. DNA lesions remain open for a longer time in the case of a decreased DNA-repair capacity, thus, the prolonged DNA-repair plays a major role in the prolongation of MD /13, 14, 23/. In the case of a missing repair capacity as in X-ray irradiated ataxia teleangiectasia FBs, no, or reduced MD was reported /6/.

The present results indicate that chemical mutagens can induce a measurably increased MD in sensitive cells, in accordance with the data on chemically induced SCE /10/. Lymphocytes from the donor with Down's syndrome were more sensitive than fibroblasts. The reported reduced DNA-repair capacity in cells with 21 trisomy /1, 4/ can explain the results obtained in Down's lymphocytes.

Acknowledgements: The author is thankful to K. Lapis and B. Szende professors for auspices and valuable criticism; and Mrs Mária Csorba for excellent technical assistance.

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Acta Medica Hungarica, Vol. 50/1—2, pp. 117—130 (1994)

EXPERIMENTAL STUDY

TIME-COURSE CHANGES IN PANCREATIC LABORATORY AND MORPHOLOGIC PARAMETERS IN TWO DIFFERENT ACUTE PANCREATITIS MODELS IN RATS

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(Received: June 25, 1994)

The aim of this work was to study in rats the temporal course of laboratory parameters and morphologic features in acute pancreatitis induced by cholecystokinin octapeptide (CCK-8) or by a closed duodenal loop. Pancreatitis was induced either with an overdose of CCK-8 (3 x 75 μg/kg at 1 h intervals) or by ligation of the duodenum on both sides of the bilio-pancreatic duct. The animals were examined at 0, 2, 4, 8, 16 and 24 h after AP induction. In CCK-8-induced acute pancreatitis, the pancreatic weight/body weight ratio (8.2 ± 1.1 mg/g) and the amylase level (44.8 ± 7.5 x 10^3 U/ml) were significantly increased vs. the controls (4.5 ± 0.8 mg/g and 3.3 ± 0.2 x 10^3 U/ml, respectively) 2 h after the intervention. The plasma CCK was significantly increased at 4 h (4.55 ± 1.7 pM) and remained elevated thereafter. The tissue malonyldialdehyde concentration was significantly elevated at 8 h (0.28 ± 0.07 μmol/mg pancreas) vs. the controls (0.20 ± 0.02 μmol/mg pancreas). In closed duodenal loop-induced acute pancreatitis, the ratio pancreatic weight/body weight steadily increased during the study; it reached its maximum level at 24 h (7.1 ± 0.5 mg/g) vs. the sham-operated control (4.8 ± 0.9 mg/g). The serum amylase level was significantly elevated at 2 h (47.1 ± 9.3 x 10^3 U/ml), and then decreased steadily. Plasma CCK values were significantly higher than the controls throughout the study. A significant increase in the tissue malonyldialdehyde concentration (0.94 ± 0.15 μmol/mg vs. 0.20 ± 0.01 μmol/mg pancreas) appeared at 4 h. Our data indicate that in CCK-8-induced acute pancreatitis the laboratory signs of pancreatitis are most expressed at 4 h, whereas the morphologic changes culminate 8 h, following the last CCK injection. In closed duodenal loop-induced acute pancreatitis, the histologic findings showed a progressive deterioration. Endogenous CCK and oxygen-derived free radicals seem to play a role in the pathogenesis of both types of acute pancreatitis.

Keywords: Cholecystokinin-octapeptide, acute pancreatitis, closed duodenal loop, tissue malonyldialdehyde, plasma cholecystokinin

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Abbreviations: CCK-8 = cholecystokinin-octapeptide, CDL = closed duodenal loop, AP = acute pancreatitis, MDA = malonyldialdehyde, pw/bw = pancreatic weight/body weight

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0236-5286/94/$ 4.00

Introduction

The two main etiological factors in the development of human acute pancreatitis (AP) are cholelithiasis and alcohol consumption /18/. According to the theory most generally accepted at present, a secretory blockade (colocalization of the lysosomal enzymes and digestive proenzymes) resulting in an autolysis of the pancreas in both cases /18/. Despite numerous experimental and clinical results, the pathomechanism of AP has not yet been established in detail /2, 8/.

A number of recent studies have suggested that the local, and especially the systemic effects of AP may be mediated in a common pathway (oxygen-derived free radicals, cytokine cascade), irrespective of the initiating stimulus /14, 17/. Sanfey et al. /14/ demonstrated the pathogenetic role of oxygen-derived free radicals. Agents which act as scavengers have been found to reduce the severity of experimental AP /12/.

CCK has also been supected to play a role in the pathogenesis of various pancreatic disorders /21/. In 1977, Lampel et al. demonstrated that high doses of CCK-8 induce edematous AP in the rat /6/. CCK-8 has been found to worsen morbidity and mortality in experimental AP caused by intraductal injection of bile salts in rats /5/. The literature is poor in data are concerning the pathogenetic role of the endogenous plasma CCK level in AP /19/.

The aim of this study was to examine the biochemical and morphological alterations in CCK-8-induced and biliary AP models in order to clarify the putative roles of endogenous CCK and lipid peroxidation in the pathogenesis of these pancreatitis models.

Materials and Methods

Male Wistar rats weighing 280-330 g were used throughout. The animals were kept at a constant room temperature (27 ^{o}C), with free access to water and a standard laboratory chow (LATI, Gödöllő, Hungary).

Experimental protocol

For both models, animals were segregated into 6 groups, with 6 rats in each, and treated as listed below.

CCK-8-induced AP model

The animals received 75 µg/kg CCK-8 (synthesized by B. Penke, Department of Medical Chemistry, Szeged) s.c. three times at hourly intervals /7/. Groups of 6 rats were sacrificed 0, 2, 3, 8, 16 or 24 h following the last CCK-8 injection. Control animals (n = 6/group) received saline s.c. and were sacrificed at the same times.

CDL-induced AP

In rats laparotomized under ether narcosis a CDL was crated by ligating the duodenum at two points on either side of the common bile duct on a plastic tube inserted into the duodenum /11/. The blood supply to the duodenal CDL was carefully preserved. The CDL was 2.0-2.5 cm in length. After the procedure, the abdomen was closed. In the control rats, a midline laparotomy was performed.

The rats were killed by exsanguination through the abdominal aorta 0, 2, 4, 8, 16 or 24 h after the ligation of the duodenum (n = 6/groups). The pancreas was carefully removed and weighed.

Assays

Serum samples were stored at -20 °C for subsequent analysis of amylase activities by the Phadebas test method /3/ and of MDA activities according to Slater's method /16/.

Plasma CCK-like bioactivity was measured by the highly specific and sensitive bioassay described by Liddle et al. /9/. CCK was extracted from 2 ml of plasma by adsorption on octadecylsilylsilica cartridges (Sep-Pak, Waters Assoc.) and eluted in ethanol and trifluoroacetic acid. The quantitation of CCK in the extracts was based on its ability to release amylase from rat pancreatic acini prepared with collagenase. The bioactivity of CCK was compared with a standard curve, and the results were expressed in pM CCK-8 equivalents. The assay is sensitive even to plasma levels as low as 0.5 pM.

The development of pancreatic edema was measured by comparing the pancreas weight obtained immediately after the animal's death (wet weight) with that of the same sample kept at 150 °C for 48 h (dry weight) as described by Ohshio et al. /13/. The ratio pancreatic wet weight/body weight (pw/bw) was calculated in each case.

Histologic examination

A fragment of the pancreas was fixed overnight in 10% neutral formaldehyde solution for haematoxylin and eosin staining and for histological study by light microscopy. For electron microscopy, tissue samples were fixed in a modified Karnowsky's solution (810 mOs), and the semithin sections were stained with toluidine blue. The sections were examined by a pathologist who was unaware of the treatment modalities, and the histological alterations were evaluated on a semiquantitative score system, graded on a scale of 0 - ++++, as minimal to maximal changes /23/. Zero indicated no change. +, ++ and +++ indicated that about 25%, 50% and 75% of the cells, respectively, were affected, and ++++ indicated that practically all the cells were affected. For interstitial lesions the following scoring system was applied: 0 = no change, + = mild interstitial oedema, ++ = mild interstitial oedema with inflammatory cells, and +++ = interstitial oedema with haemorrhagic lesions.

Statistical analysis

Results were expressed as means ± SEM. Experiments were evaluated statistically with Student's t-test for paired or unpaired values, as appropriate. P values < 0.05 were accepted as significant.

Results

Results on the CCK-8 model

75 μg/kg of CCK-8 administered s.c. 3 times at hourly intervals significantly increased the pancreatic weight and water content of the organ relative to the controls. The ratio pw/bw was greatly increased after the induction of AP and reached its peak 4 h after the last CCK-8 injection (Fig. 1). After 4 h, the ratio pw/bw decreased continuously, and it had normalized by 24 h (Fig. 1).

The serum amylase activity rose steadily up to 24-fold by 4 h after the last CCK-8 injection (Fig. 2). It had reached the control level by 16 h (Fig. 2).

The plasma CCK bioactivity was significantly increased at 4 h and remained elevated thereafter (Fig. 3).

The tissue MDA concentration was slightly but significantly elevated 8 h (Fig. 4).

Macroscopic study of the pancreata in the CCK-8-treated group revealed interstitial oedema. Microscopic examination demonstrated a moderate diffuse parenchymal degeneration, including vacuolation and focal necrosis (dark cells) within the acinar cells. These morphological changes were most expressed (++ or +++) at 8 h and gradually decreased thereafter (Figs 5—7). The interstitium remained virtually unchanged (grade: 0).

Results on the CDL model

Both the pancreatic weight and the pancreatic water content were increased after the rats with CDL AP. The ratio pw/bw was significantly above the basal value at 2 h and increased continuously during the experiment; it reached its maximum level at 24 h (Fig. 8).

The serum amylase activity attained its peak level at 2 h, and afterwards decreased continuously (Fig. 9).

The plasma CCK bioactivity was significantly increased 2 h after the onset of AP, and reached its highest level at 4 h (Fig. 10).

The tissue concentration of MDA was 5-fold the control value at 4 h and then declined abruptly (Fig. 11).

On visual inspection, the pancreas seemed to be oedematous with occasional haemorrhages. Microscopic examination revealed an acute inflam-

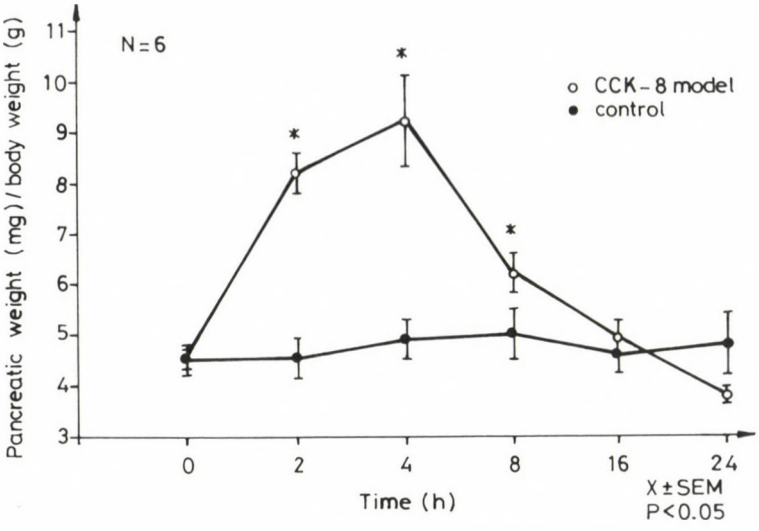

Fig. 1. Ratio pancreatic weight/body weight (pw/bw) in CCK-induced AP. Rats received 3 x 75 µg/kg of CCK-8 at 1 h intervals (open circles), or the same volume of saline (control cases: filled circles); they were sacrificed at the times indicated. The pancreatic wet weight and body weight were measured and the ratio pw/bw was calculated. Means ± SEM for groups of 6 rats are shown. *Significant differences from the control values ($P < 0.05$)

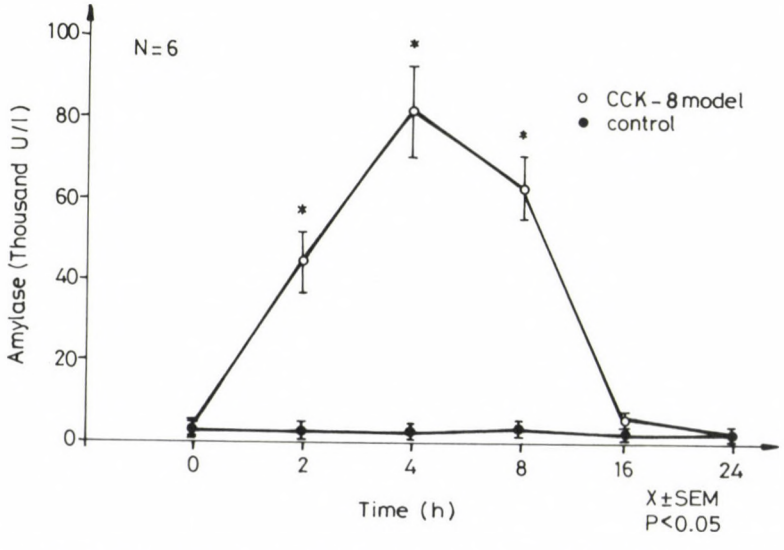

Fig. 2. Serum amylase activity in CCK-induced AP. Rats received the same volumes of CCK-8 (open circles) or saline (filled circles) as indicated in Fig. 1, and were sacrificed at the times indicated. Means ± SEM for groups of 6 rats are shown. *Significant differences from the control values ($P < 0.05$)

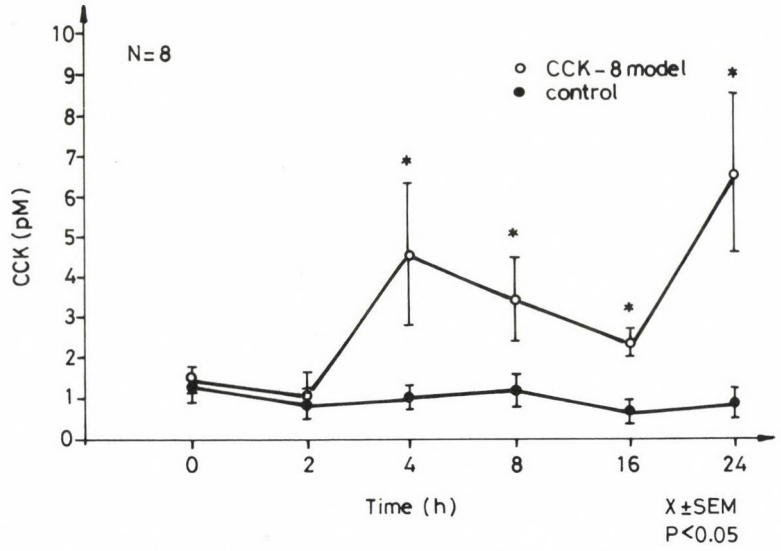

Fig. 3. Plasma CCK levels in CCK-induced AP. Rats received the same amount of CCK-8 (open circles) or saline (filled circles) as indicated in Fig. 1, and were sacrificed at the times indicated. Plasma CCK was measured by bioassay as described in detail in the text. Means ± SEM for groups of 6 rats are shown. *Significant differences from the control values ($P < 0.05$)

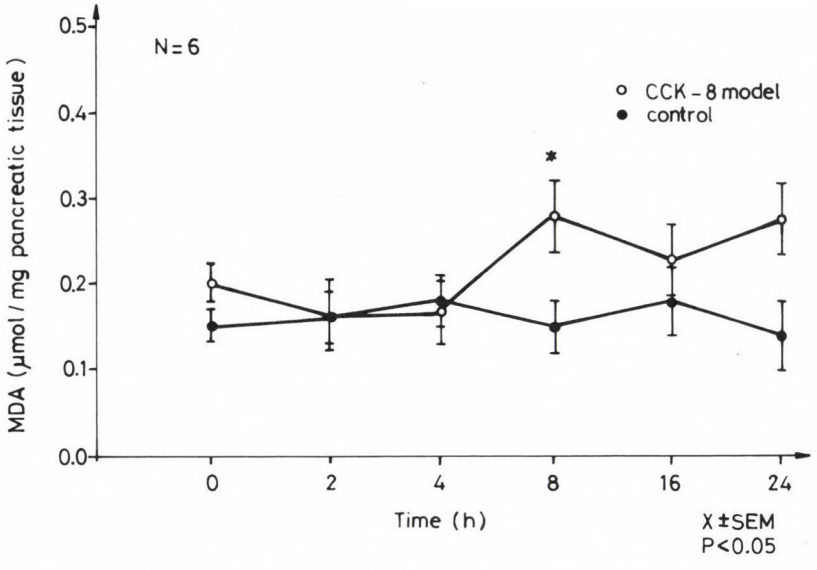

Fig. 4. MDA activity in CCK-induced AP. Rats received the same amount of CCK-8 (open circles) or saline (filled circles) as indicated in Fig. 1, and were sacrificed at the times indicated. Means ± SEM for groups of 6 rats are shown. *Significant differences from the control values ($P < 0.05$)

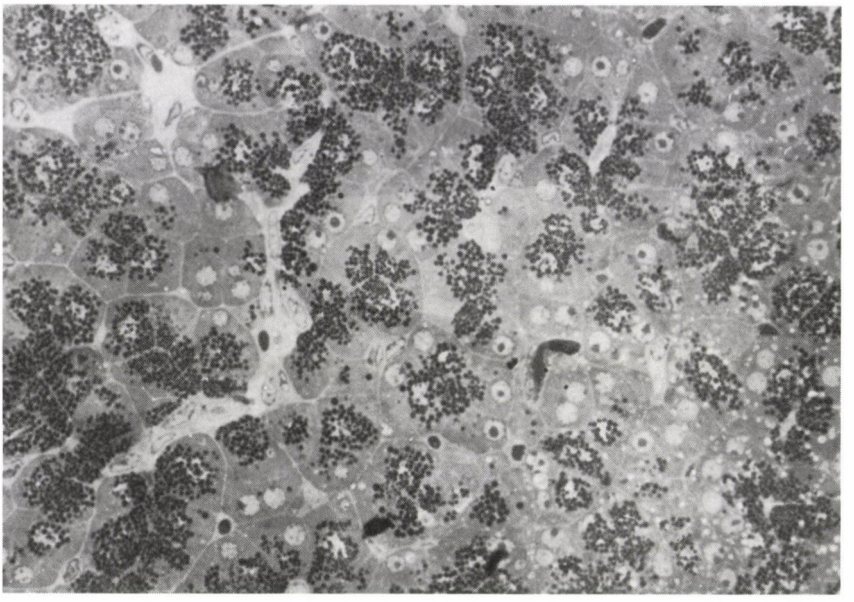

Fig. 5. Light microscopy of a control rat pancreas. Normal structure of pancreas with distinct cellular outlines and globular vesicular nuclei. The apical cytoplasm contains fine granules (semithin section, toluidine blue, x40)

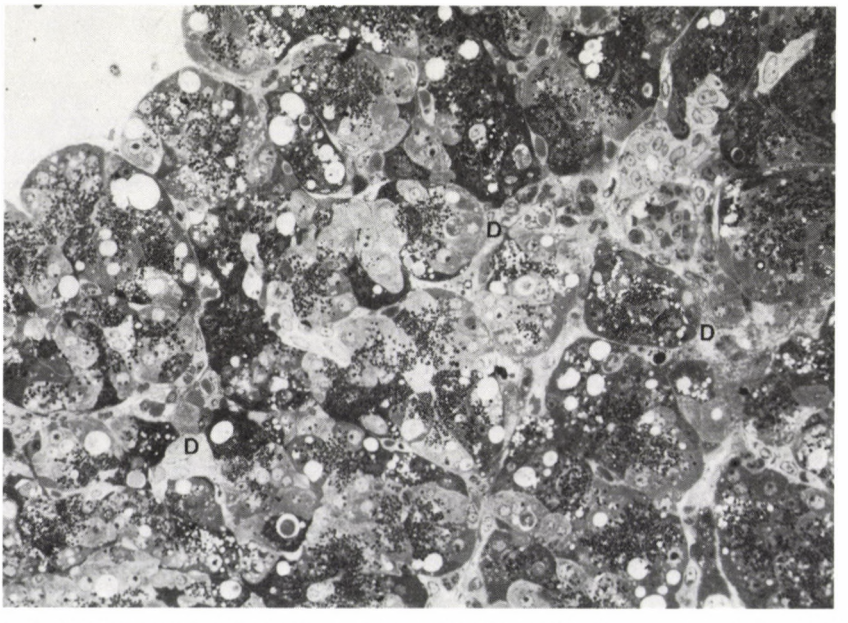

Fig. 6. In CCK-induced AP marked acinar cell vacuolation and focal necroses — dark cells (D) — are visible after 8 h (semithin section, toluidine blue, x25)

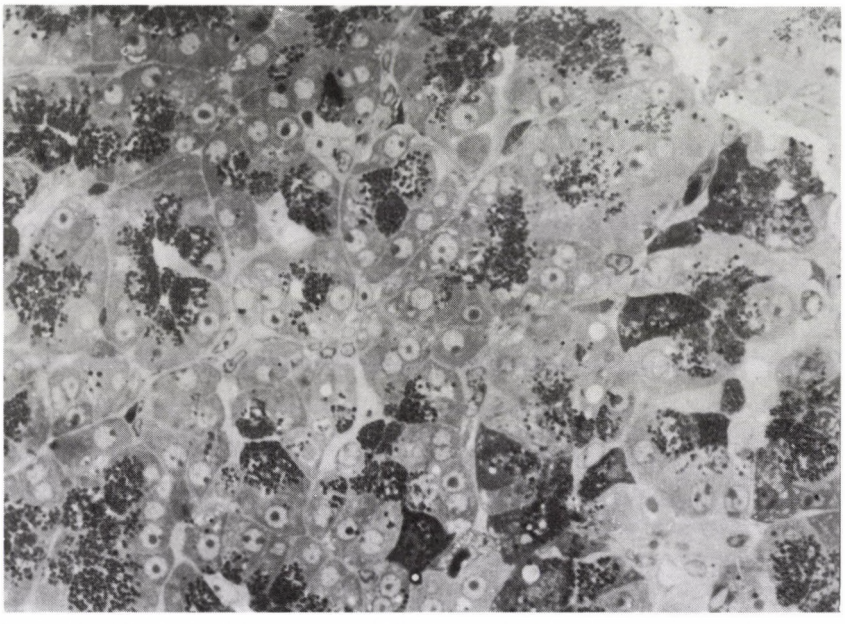

Fig. 7. In CCK-induced AP vacuoles are missing after 24 h, only some dark cells are visible (semithin section, toluidine blue, x40)

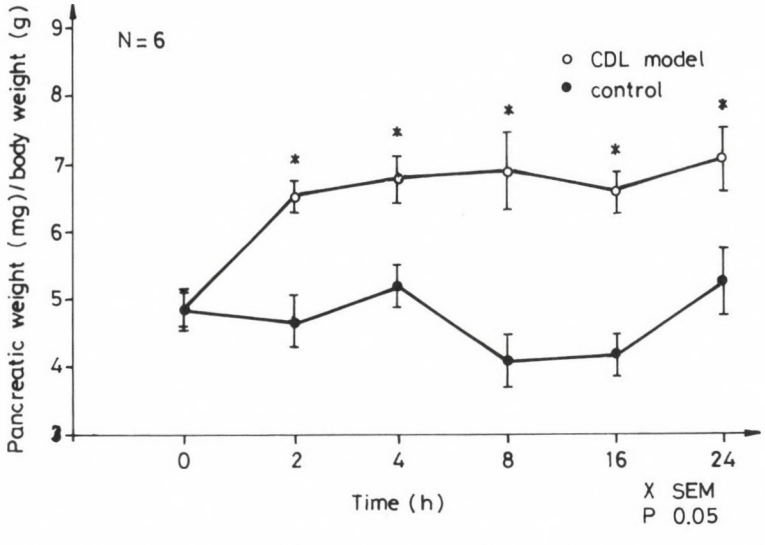

Fig. 8. Ratio pw/bw in CDL-induced AP. Test animals (open circles) and controls (filled circles) were surgically prepared and sacrificed as described in detail in the text. The pancreatic wet weight and body weight were measured and the ratio pw/bw was calculated. Means ± SEM for groups of 6 rats are shown. *Significant differences from the control values ($P < 0.05$)

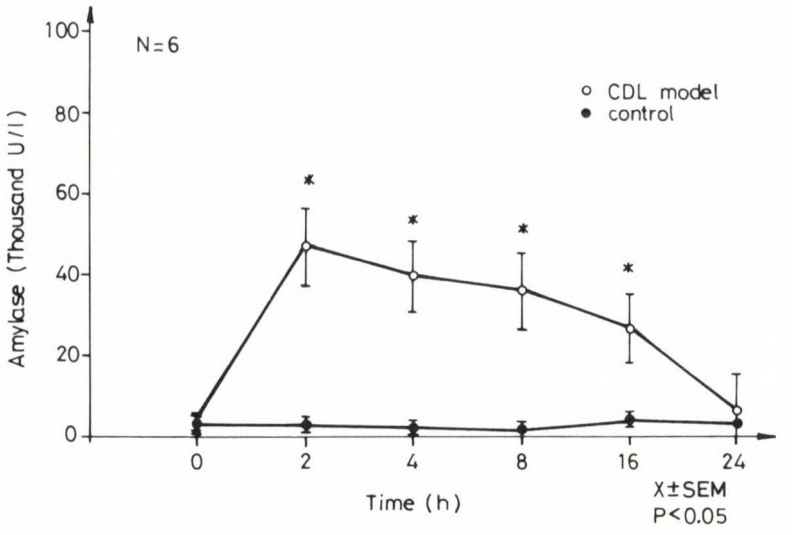

Fig. 9. Serum amylase activity in CDL-induced AP. Test animals (open circles) and controls (filled circles) were surgically prepared and sacrificed as described in detail in the text. Means ± SEM for groups of 6 rats are shown. *Significant differences from the control values ($P < 0.05$)

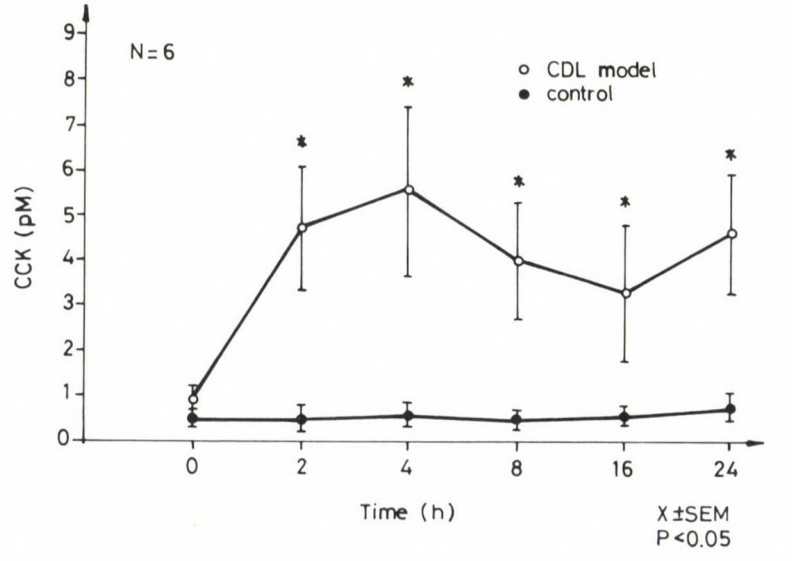

Fig. 10. Plasma CCK levels in CDL-induced AP. Test animals (open circles) and controls (filled circles) were surgically prepared and sacrificed as described in detail in the test. The plasma CCK was measured by bioassay. Means ± SEM for groups of 6 rats are shown. *Significant differences from the control values ($P < 0.05$)

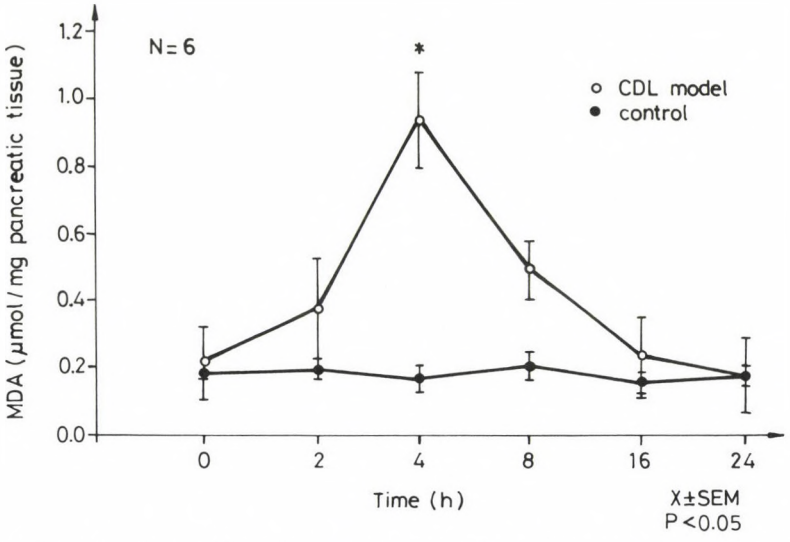

Fig. 11. MDA activity in CDL-induced AP. Test animals (open circles) and controls (filled circles) were surgically prepared and sacrificed as described in detail in the text. Means ± SEM for groups of 6 rats are shown. *Significant differences from the control values ($P < 0.05$)

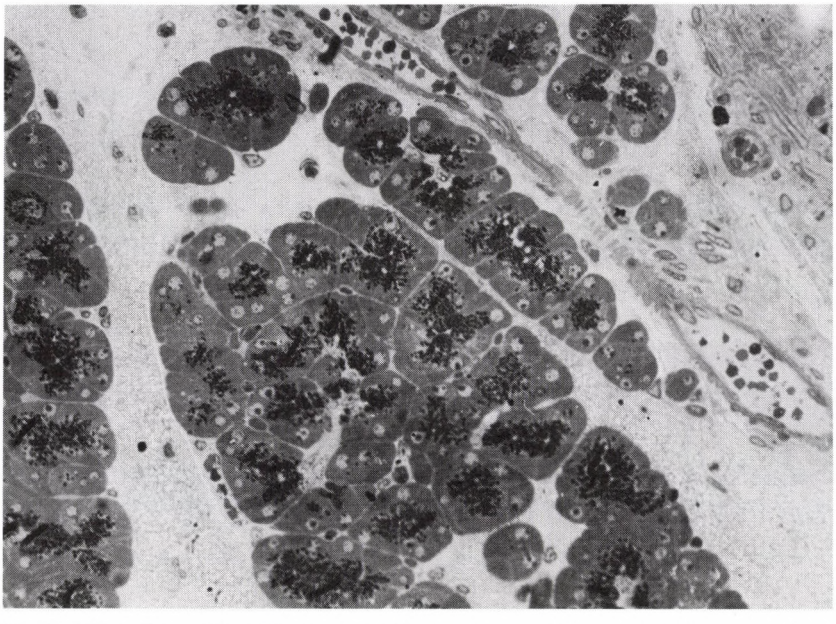

Fig. 12. In CDL-induced AP enlarged interstitial space with dilated blood vessels can be detected after 8 h. The acinar cell structure is intact (semithin section, toluidine blue, x25)

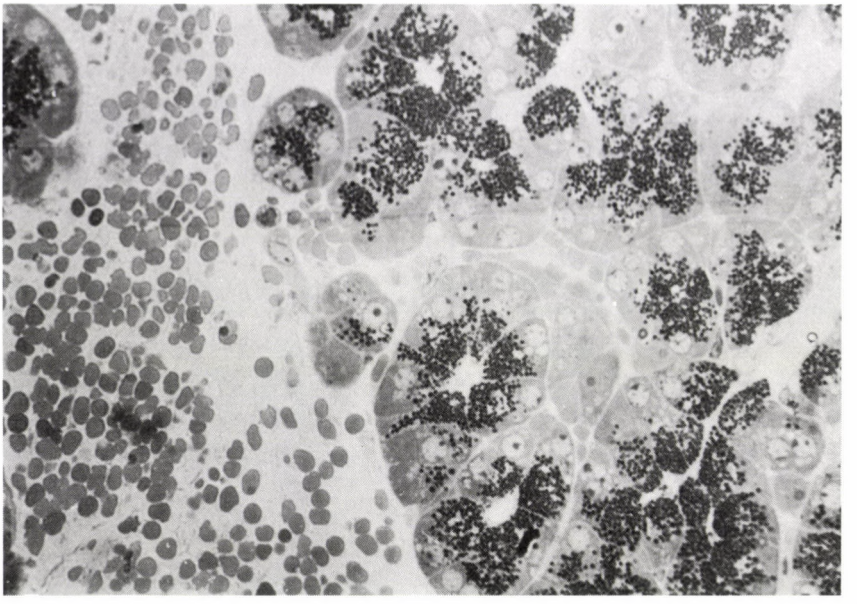

Fig. 13. In CDL-induced AP an acute haemorrhagic inflammation is clearly visible after 24 h without lesion of parenchymal cells (semithin section, toluidine blue, x40)

matory infiltration in the interstitium. Large amounts of fluid, neutrophils and red blood cells were seen to be leaking out of the vessels. These histologic alterations already existed at 8 h (grade: +), and further increased (grade: ++ - +++) during the experiment, resulting in acute haemorrhagic inflammation by the end of the study (Figs 12—13). The acinar cells remained nearly normal (grade: 0 - +) (Fig. 13).

Discussion

Because of the typically rapid onset of clinical AP and the poor accessibility of human pancreatic tissue, the pathophysiology of AP have examined mainly on experimental models of the disease /1/. Since 1977, when Lampel et al. described CCK hyperstimulation-induced AP, this model has served as a prototype of oedematous pancreatitis /6/. Another clinically relevant and widely accepted model of experimental AP is biliary-type AP, in which the biliopancreatic duct is obstructed surgically with a simple ligation /1/ or through formation of a CDL /11/.

In both models, a blockade of exocytosis from acinar cells at a time when the pancreatic enzyme synthesis is unimpaired ("pancreastasis") is the earliest intraacinar aberration. The pancreatic enzyme levels in the blood rise abruptly, the acinar cell cytoplasm displays vacuoles containing digestive and lysosomal enzymes (in CCK-induced pancreatitis) and focal interstitial haemorrhage appears (in biliary-type AP). In spite of the considerable number of publications concerning the pathomechanism of AP, there is no ready explanation for the common pathomechanism of the disease /1/.

CCK and other gastrointestinal hormones have been supposed to participate in the pathophysiology of experimental AP /5/. Recent studies with specific CCK receptor antagonists have demonstrated a marked protective effect of these agents (i.e. CR-1392, CR-1409 and L-364, L-718) in different AP models /10, 13, 24/. These results provided further evidence on the role of CCK in AP. However, there are only few data on the plasma CCK level during the early phase of AP /19, 22/.

Our data indicate that in CCK-induced AP the laboratory signs (ratio pw/bw and serum amylase levels) are the most expressed at 4 h, and they are decreased at the end of the study. In this model, the plasma CCK values were also significantly elevated at 4 h and remained so throughout the study. In spite of the constantly elevated plasma CCK level, the morphological signs of AP were most marked at 8 h, and then diminished continuously. This indicates that the elevation of the plasma CCK in this model may have been a consequence of the secretory blockade and a negative feedback mechanism. The lack of a correlation between the plasma CCK level and the morphologic signs of AP demonstrates that the endogenous plasma CCK plays only a supportive role in the pathogenesis of oedematous AP.

In the biliary type of AP, the laboratory parameters of pancreatic inflammation are already pronounced 2 h following CDL formation. The rise in plasma CCK was quicker (at 2 h) and more expressed than in CCK-induced AP. In this model, morphological signs started at 8 h in the interstitium, and progressive haemorrhagic acute inflammation developed without marked acinar cell damage.

A number of data are available concerning the crucial role of oxygen-derived free radicals in the development of experimental AP /4, 15/. Direct increases in lipid peroxidation products and protective effects of different scavengers (i.e. superoxide dismutase, dimethylsuphoxide and allopurinol) in AP models have been described /12/. In our study, the increase in a lipid peroxidation product (MDA) in the course of biliary AP displays a strong

correlation with the laboratory signs of pancreatic inflammation, In CCK-induced AP, only a slight but significant increase in plasma MDA activity was found at 8 h. The significant elevation of the plasma MDA activity as a result of the increase in lipid peroxidation demonstrates that oxygen-derived free radicals may be involved in the pathogenesis of AP in both models.

In conclusion, our results have proved that repeated administration of CCK-8 causes only a mild oedematous, interstitial type of AP. By the end of the study, the pancreatic architecture was totally restored. In biliary-type AP, however, the pancreatic histological picture progressively deteriorated and severe haemorrhagic signs developed. Besides the supportive role of endogenous CCK, other mechanisms (involving oxygen-derived free radicals, for instance) seem to be involved in the process of acute pancreatic damage.

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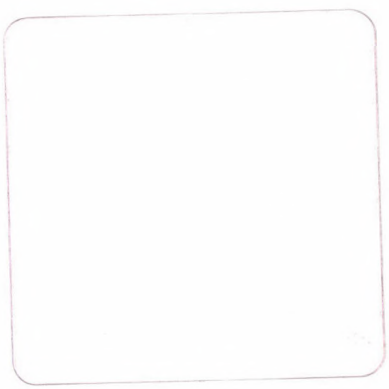

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Acta Medica Hungarica

VOLUME 50, NUMBERS 3–4, 1994

Akadémiai Kiadó, Budapest

ACTA MED. HUNG. 50 (3–4) 133–296 (1994) HU ISSN 0236–5286

ACTA MEDICA HUNGARICA

A QUARTERLY OF THE HUNGARIAN ACADEMY OF SCIENCES

Acta Medica publishes reviews and original papers on clinical and experimental medicine in English. *Acta Medica* is published in yearly volumes of four issues by

AKADÉMIAI KIADÓ
Publishing House of the Hungarian Academy of Sciences
H-1117 Budapest, Prielle K. u. 19–35

Manuscripts and editorial correspondence should be addressed to the Managing Editor:

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Subscription information

Orders should be addressed to

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Subscription price for Volume 50 (1994) in 4 issues US$ 84.00, including normal postage, airmail delivery US$ 20.00.

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*Lectures presented at the Meeting of the Korányi Sándor Society, Budapest, April 1994

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PRESENT RESEARCH TRENDS IN CANCER CHEMOTHERAPY

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(Received: September 23, 1994)

Those research trends which are currently in the focus of interest of various research groups are summarized. The main tendency is to look for new molecular targets and to synthesize new antitumour drugs. Moreover, new research concepts emerge out of which differentiation induction, inhibition of MDR and study of apoptosis seem to be promising. Among clinical approaches megachemotherapy, neoadjuvant treatment and progress in supportive therapy are the main research directions. Last but not least, quality of life issues of the cancer patient are of particular importance.

Keywords: Chemotherapy, new research trends, redifferentiation, apoptosis, megatherapy, adjuvant therapy

Cancer chemotherapy is a fairly new therapeutic modality of malignancies. Its origin goes back to World War II /3/. Since that time four periods can be distinguished in the development of drug selection (Table I) /2/.

Each period sets different objectives. The goals are sometimes very closely related to each other and are reviewed in Table II.

Recently, the revolutionary findings in molecular biology have given an enormous push to the development of drug therapy. There are new targets as well as new agents. The targets in the cancer cell are numerous. The major trends are shown in Table III /7/.

Based on these concepts, a series of investigational drugs have been produced. These are either analogues of existing, effective antitumour compounds or completely new chemical structures.

Table IV shows a summary of those agents which are currently in phase I or II trials /8/.

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Table I

History of cancer chemotherapy. Drug selection

Period	Years	Models used for drug selection
Empirical	1945—1959	Tissue cultures Transplantable rodent tumours
Rational	1960—1979	Nude mice, athymic mice, other xenografts
Complex	1980—1989	Human tumour cell lines Transgenic mice
Molecular	1990—	Inhibition of gene activity, immune blotting, PCR*

*PCR = Polymerase Chain Reaction

Table II

Objectives of cancer chemotherapy

Periods	Objective
Empirical	Antitumour effect: remission induction, prolongation of survival, tolerable toxicity
Rational	Curative effect of sensitive tumours, decreased toxicity
Complex	Curative effect with other therapeutic methods, less toxic drugs
Molecular	Targeted chemotherapy of genes, redifferentiation, improved quality of life

The cytotoxic analogues were synthesized in order to possess less toxic substances with activity equal or even larger with a borader tumour spectrum than the parent compound.

Since this goal was only partly achieved, many new drugs have also been produced to explore their antineoplastic effect. These are listed in Table V /4/.

These agents are still subject to research. Nevertheless, some of them (e.g. taxanes) have new mechanisms of action. Accordingly, it can be expected that their clinical application might prove to be useful in case their toxicity can be overcome.

Table III

New targets for cancer chemotherapy

Level	Approach
DNA, RNA, histone, protein synthesis	Topoisomerase inhibitors, antisense molecules, antiligase activity, etc.
Gene activity	MDR*, altered oncogene and suppressor gene activity inhibition
Cell division	Inhibition of tubulin assembly and mitosis
Organism	Antimetastatic therapy

*MDR = Multidrug Resistance

Table IV

New cytostatic analogues in phase I-II trials

Chemical Group	New derivatives
Anthracyclines	Epi-, ida-, iodo-, pira-, hydroxyrubicine
Anthracenedions	Mitoxantrone, Losoxantrone
Anthrapyrazoles	Piroxantrone, BBR 2778
Oxazaphosphorines	Ifosfamid, Trofosfamid, Mafosfamid
Vinca alcaloids	Vindesine, Vinorelbine
Camptothecins	Topotecan, Irinotecan, Intoplicin
Platinum derivatives	Carbo-, ipro-, oxo-, lobo-, amino-, cyclo-, ormaplatin
Nitrosoureas	Fotemustin, cistemustin
Naphthylureas	Suramin
Antimetabolites	Trimetrexate, edatrexate, AZT, AZC, gemcitabin, 2-chloro-2-fluor-adenosine

There are also new concepts which have resulted in progress of therapy of malignant diseases. The major research approaches are enumerated in Table VI.

What is differentiation induction? The assumption that a premalignant or malignant cell might be reintegrated into its normal environment instead of its killing has been confirmed. For example, oral leukoplakias might be healed by retinoic acid derivatives /12/. Those agents which are capable of stimulating this "redifferentiation" process are called "differentiation inducers". Many such substances exist. Table VII lists the most important redifferentiating agents /16/.

Table V

Investigational antitumour drugs

Groups of origin	Drugs
Taxanes	Taxol, taxotere
Antiproteases	Distamycin
Polyamine inhibitors	Deoxyspergualin, PT-523
Antilipases	Crotoxin
Plant derivatives	Ipomeanol, flavonic acid, elaginin
Barbiturates	Merbaron
Aziridines	Imexon
Chinons	Chlorochinoxalin, ansamycin
Heavy metal complexes	Titanium, Ruthenium complexes
Metal complexes	Tellurates

Table VI

New concepts in drug therapy

Differentiation induction (redifferentiation)
Enhancement of apoptosis
Inhibition of MDR
Potentiation of drug effect
Megatherapy, supportive drugs
Adjuvant (neoadjuvant) therapy

The mechanism of action of differentiation inducers is not clear. At least three different explanations exist: (1) influence exerted upon cell surface molecules, (2) enhancement of ligase type activity, and (3) production of various enzymes responsible for subcellular metabolic activity.

Another substantial research topic is cell death (apoptosis). Normally, there is a balance between cell reproduction and cell loss. Such balance exists between new and dyeing cancer cells as well. Any effort which is directed towards the enhancement of cell death, distorts this equilibrium. Consequently, if more malignant cells disappear than the amount in which they are produced, the tumour is in a shrinking stage. The result may also be a complete tumour disappearance ("regression").

Several research ways exist for influencing cellular metabolism and inducing apoptosis. Table VIII reviews certain avenues /14/.

Basically, agents inducing apoptosis might be either molecules exerting activity selectively against cell surface adhesion proteins (autocrine

Table VII

Redifferentiating agents

9-cis-transretionoic acid	Vitamin D_3
13-cis-transretinoic acid	Tamoxifen
Fenretinid	Bisacetamide derivatives
Tocopherol	Phorbol derivatives

Table VIII

Apoptosis induction

Autocrine mechanisms	Antiadhesion molecules (anti-adherrin, antilectins, anti-fibronectin, etc.)
Paracrine mechanisms	Antimetastatic therapy (bryostatin, etc.)

or intrinsic factors) or new chemical structures influencing cell death (paracrine or extrinsic factors). Some of them are increasing cell degradase activity by a series of various metabolic steps.

Great excitement was attached to the discovery that certain glycoproteins (P170 proteins) may accumulate in tumour cell membranes, thus inhibiting drug uptake. As a result, the malignant cell becomes "resistant" to any cytostatic therapy. This phenomenon may manifest itself primarily as a cell inbuilt property, or, secondarily, after a certain period of treatment. It represents a major obstacle to the definitive success of cancer chemotherapy. At least a half of the existing drugs show this "multidrug resistance" (MDR). MDR is an overexpression of three different genes, resulting in an excess of glycoproteins in the cancer cell embrane. MDR is probably the most important type of drug resistance /10/.

Any effort aimed at overcoming resistance means a success in therapy. Therefore, several agents with various mechanisms of action have been produced to inhibit MDR. These substances are shown in Table IX.

Based upon pharmacokinetic data mainly published in the last decade the study of mechanism of action of several antitumour compounds has been renewed. Various substances have been found which are capable of increasing the concentration of different enzymes, thus, preparing the target for an increased effect of the cytostatic drug. These "potentiating" substances are

Table IX

Agents inhibiting MDR

Calcium channel blockers	Verapamil, nifedipin, nomodipin, guldipin, dexniguldipin
Sporins	Cyclosporin, staurosporin
Camptothecins	CPT-11, irinothecan
Podophyllins	Etoposide, tenoposide

Table X

Potentiating drugs under current clinical investigation

Drugs	Malignancies
Leucovorin	Colorectal cancer
Zidovudin	Gastric cancer
Dipyridamol	Breast cancer
Acivicin	Head and neck cancer

non-cytotoxic drugs. As an example, initially drug "potentiation" was used to enhance fluoropyrimidine activity by leucovorin. Table X demonstrates compounds possessing potentiating effect /13/.

Actually, various antimetabolites and potentiating agents are under clinical investigation in different malignant tumours.

The dosage of established drugs has also been reinvestigated. The term "megatherapy" means very high doses of effective antineoplastic drugs. This approach aims at destroying —with the aid of large concentrations of cytostatic drugs — those tumour cells which are insensitive to standard doses. This procedure became possible because promising advances were made in supportive therapy. The major results are listed in Table XI /9, 11/.

Last but not least, methods of adjuvant therapy has been revisited in the late eighties in order to find an established place for cytotoxic treatment within the frame of complex therapy. Metaanalysis showed that postoperative adjuvant chemotherapy of premenopausal N^+ breast cancer patient with various antitumour drugs is useful while postmenopausal women of the same category require postoperative hormonal treatment. Several malignancies were also objects of successful postoperative adjuvant chemotherapy. Apart from this approach preoperative adjuvant chemotherapy was initiated to im-

Table XI

Advances in supportive therapy

Type of toxicity	Therapeutic approach
Haemopoietic	— BM-transplantation (heterologous, autologous)
	— Progenitor cell infusion
	— Growth factor therapy (H-EPO, G-CSF, GM-CSF, IL-3)
Antiemetic	Antiserotonins (ondan-, tropi-, granisetron)
Uroepithelial	MESNA
Cardiac	WR-2721 ?
Antiallopecic	Tellurates ?
Antihepatic	Thiol derivatives ?

Table XII

Adjuvant chemotherapy

Type	Malignancy
Preoperative (neoadjuvant)	Oral cancer Laryngeal cancer Oesophageal cancer Osteosarcoma
Postoperative (data from metaanalysis)	
— compulsory	Premenopausal N^- breast cancer, Postmenopausal N^- breast cancer with hormones Wilms tumours, neuroblastoma, rhabdomyosarcoma
— optional	Gastric carcinoma in situ Colorectal cancer

prove the results of surgical intervention as well. Table XII is a review of the most important research trends /1/.

The methods of adjuvant therapy performed by immunological methods has not yielded any solid data. There is still a debate whether the administration of immunostimulants in colorectal cancer or melatoma is useful or not. Clinical investigations are in progress.

In both experimental and clinical research, it is the cancer patient who is in the focus of interest. He is the suffering individual and if there is no cure for him at least he can be relieved from its burdening or painful symptoms /5/. His quality of life has to be insured by all means. Every step in research has to have the goal to assist him in preserving this quality. This effort is now in the centre of interest of dedicated researchers. I am hopeful that we will be able to fulfil this task in the near future.

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Acta Medica Hungarica, Vol. 50/3—4, pp. 141—151 (1994)

THE ROLE OF CT IN THE TREATMENT PLANNING OF RADIOTHERAPY OF MALIGNANT TUMOURS

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(Received: September 23, 1994)

The advantages of and the information given by CT examinations in the proper and valid treatment planning of radiotherapy of malignant tumours are discussed. Without individual and thoughtful application of CT before and during radiotherapy the chances of cure are significantly reduced. Radiotherapists must be familiar with this imaging method.

Keywords: CT, therapy planning, radiotherapy, malignant tumours

Survey

Our aim in the radiotherapy of malignant tumours is to decrease the number of clonogenic tumour cells to such a level that the defence mechanisms of the immune system can alone overcome them. This critical number of clonogens is supposed to be about 10^6 cells. Continuous regression of the tumour is achieved by a complex process initiated by absorption of radiation energy and produced by various biochemical, radio- and tumourbiological as well as cell-kinetic processes in tumour during irradiation. The amount of the daily dose fraction administered, as well as the total tumour dose, is of great importance, as is the homogeneity of the dose distribution in the tumour. The dose that is thought to be effective in killing tumour cells is usually associated with risks to normal tissue damage thus the given dose should never exceed normal tissue tolerance. Unfortunately, it is not possible to fulfil completely this requirement. Healthy tissues and organs lying in the path of beams unavoidably receive a certain amount of radiation energy. It is our aim to keep this amount of radiation and the unnecessarily irradiated volume of normal tissues as low as possible.

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Just like the sailor navigating between Scilla and Charybdis, there is a narrow path for the radiotherapist to walk between the necessary dose to reduce the number of clonogens and the tolerance dose of healthy tissues and organs. This narrow path, which depends on various factors, is defined as therapeutic ratio.

Similarly to the sailor, who must decide whether sealing is reasonable or not, and how to sail and on which route, the radiotherapist must consider several circumstances whilst deciding the indication of radiotherapy, viz. whether radiotherapy is indicated or not. The parameters, how to treat, by which field arrangement, by which radiation energy, dose fractions, dose distribution, etc., these details altogether are called treatment planning.

The treatment planning contains all the physical parameters which are needed to ensure the homogeneous dose distribution in the target volume. The target volume is determined by the tumour volume together with a certain amount of a surrounding volume of apparently normal, healthy tissue. This latter contains tissue that is potentially infected by tumour cells and a tissue free of microscopic lymphogenous progression. The amount of surrounding tissue potentially invaded depends on the size and histological type of the tumour. Anaplastic tumours must be irradiated by larger fields than well differentiated ones. The target volume may also contain the regional lymph node area, either because metastases are already present or the microscopic spread into the regional lymphatics is highly suspected. Obviously, the size and the shape of the volume to be irradiated varies significantly just as its relationship to adjacent organs of various radiosensitivity i.e. tolerance. It is important to irradiate the target volume as homogeneously as possible. This is true for all daily administered treatment fractions even if tumours are finally inhomogeneously irradiated. Such an inhomogeneous dose distribution gives tumour regression more effective and reduces the volume of irradiated normal tissues. It is well known that oxygen tension in the cells influences radiosensitivity. The central part of the tumour contains various amounts of hypoxic cells which are more resistant than the well oxygenated ones. At the periphery of the tumour the cells are mostly well oxygenated, thus central part needs higher dose for cell killing than its peripheral part. The size of irradiated volume can thus be decreased step-by-step during the final period of radiation therapy. This method of treatment, using shrinking fields, improves the rate of tumour control and decreases normal tissue damage. It is obvious that treatment planning is performed not only before the start of the treatment, but also before any modification that may be necessary during treatment.

It is of great importance to accurately know the localization and size of the tumour as well as its relation to the neighbouring organs. To develop a realistic treatment plan, one must possess a transversal sketch

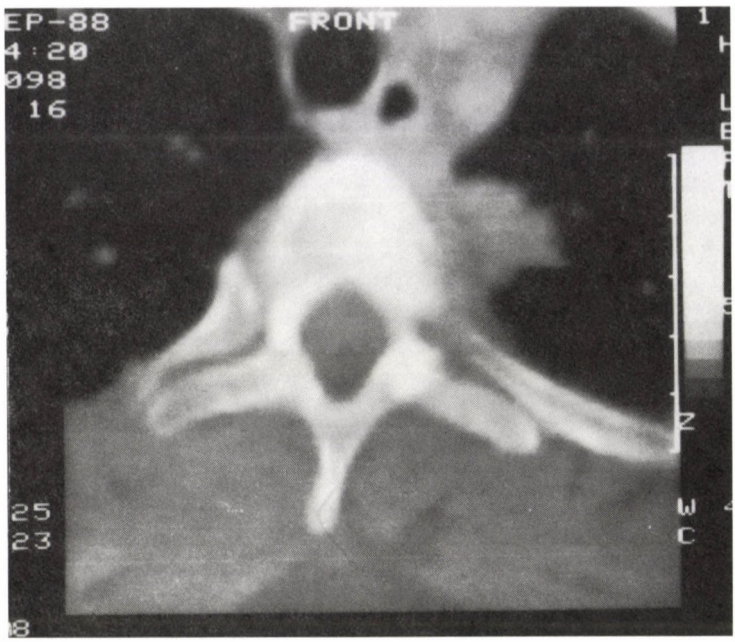

Fig. 1. Pulmonary infiltration was detected during check-up examination. The patient was symptomfree. The left border of the VII thoracic vertebra shows light moth-eaten destruction

from the body at the level of the tumour. Prior to the introduction of CT it was very difficult to get transverse sections from the body. Treatment planning based on these drawings was not only lengthy but also of low accuracy /2/. Before the CT era it was impossible by any conventional radiological method to demonstrate tumorous infiltration of soft tissues, early invasion of the bone (Fig. 1), and lymph node metastases in hidden sites (Fig. 2). Data in the literature /1/ as well as our early experiences in 1981 with CT showed that treatment plans without the use of CT required modification in 35-40% of the cases, because the size and invasion of the tumour had been underestimated. The underestimation of the target volume would have resulted in an undertreatment of some of its parts. The English literature characterizes this situation as "geographic miss" /2/, the result of underestimation of the tumour bound with increased frequency of local recurrence. Owing to the use of CT in the evaluation of the tumour this type of recurrence has become rare, now it is caused mostly by inaccurate patient set-up or by field displacement during daily treatment.

The advantages of CT in treatment planning are as follows:

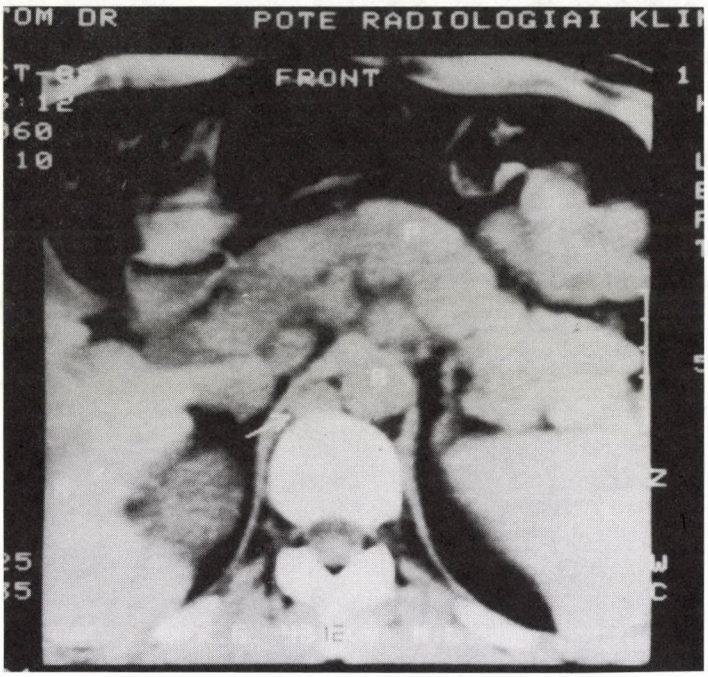

Fig. 2. CT scan demonstrates an enlarged lymph node in the retrocrural area (arrow), right to the aorta (A). This region remains hidden when conventional imaging methods or ultrasonography is used

The axial scans of the CT provide correct cross-sectional (transversal) pictures of the body with all anatomical details and of the tumour at the chosen level. There has been a debate in the literature whether CT scans should be taken after deep inspiration or expiration, always withholding breathing. In our department, CT scans are performed during normal breathing because this is the normal state during irradiation, too. The steady movement of organs due to breathing is identical during the examination and the irradiation. CT allows simple determination of the body contour, which is needed for treatment planning, but only if the gantry is wide enough even for obese patients. In this regard the CT-s operating in our country are insufficient. For this and other reasons it is recommended to equip radiotherapy centres with CT's specially designed and produced for treatment planning.

CT scans provide excellent visualization of the tumour and neighbouring organs; the absorption differences between soft tissues can be increased by intravenously administered contrast material. It is yet unknown

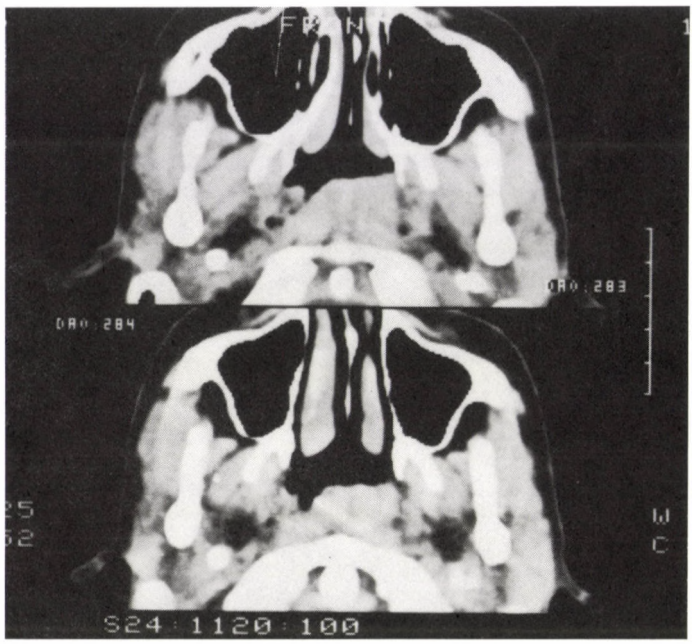

Fig. 3a. CT scan shows tumour of the nasopharynx as asymmetry on the left side of the posterior wall

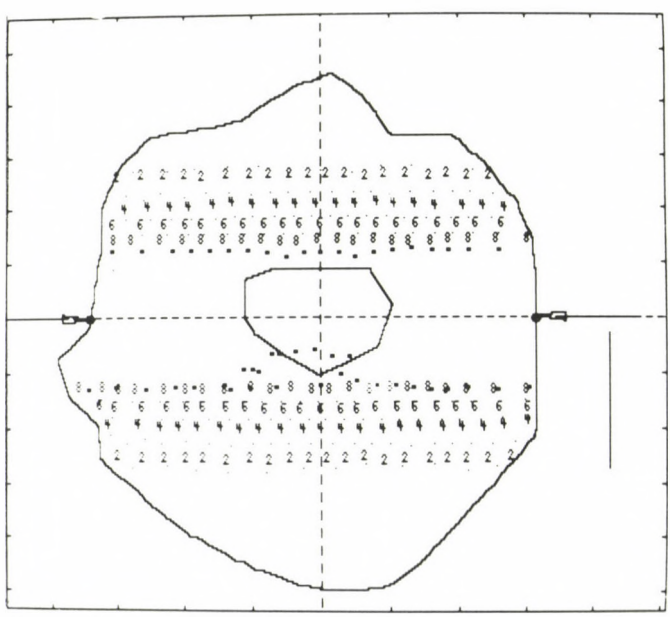

Fig. 3b. Dose distribution in the target volume

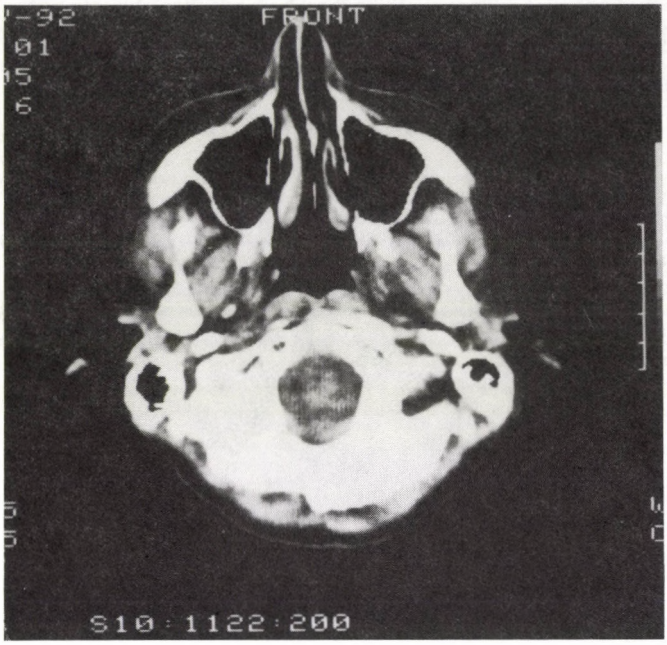

Fig. 3c. CT scan made 2 years following radiotherapy demonstrates complete regression of the tumour

whether MRI will bring about a revolutionary change in this area, similar to that caused by the introduction of the CT. It can reasonably be expected that in certain locations (brain stem, posterior fossa, spinal canal, bone marrow, etc.) MRI will allow more precise recognition of the tumour and tumorous infiltration.

Delineation of the target volume and all structures to be shielded can precisely be performed on the CT scan (Fig. 3a-b-c). The examination must be made in the same position of the patient in which irradiation will be performed. It has been well known since the introduction of CT that organs which had been thought as fixed made significant movements depending on the body's position.

The computer allows reconstructions in sagittal, frontal or any other plane. This frequently improves the determination of the shape of the target volume and its relation to surrounding organs (Fig. 4a-b). In each scan we can delineate the shape of the tissue to be irradiated. It is also possible to sum up all these marked areas and, after processing, to project the resulting area to the topogram. The projection of the volume to be irradiated made visible on the body with well recognizable relationship to fixed anatomical landmarks, is of great value in radiation-field arrangement (Fig. 5). Using specially developed sophisticated software programs we can perform a three-dimensional reconstruction of the target volume, thus, make treatment planning more accurate. Not infrequently, the scan showing the tumour surprisingly discovers tumorous manifestation in an other location, too, which in lack of clinical symptoms could have been completely hidden (Fig. 6).

The CT scan can accurately evaluate tumour regression, which is, as mentioned above, of great importance in shaping correctly the size of the radiation fields to be decreased

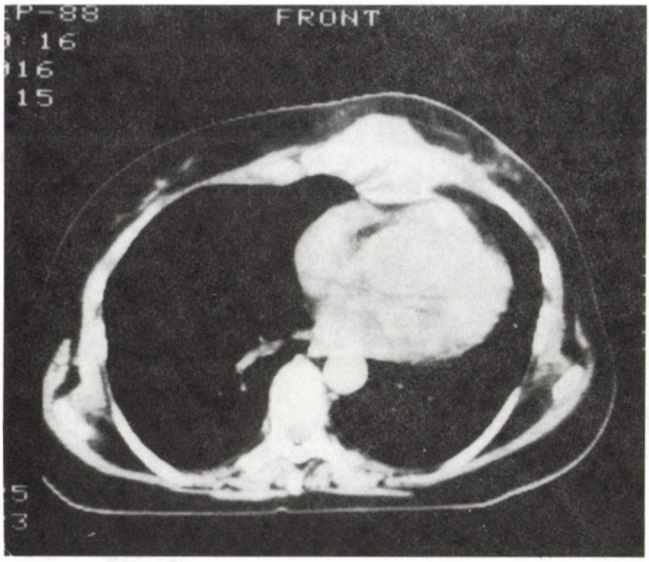

Fig. 4a. CT scan displays recurrent tumour in the left prasternal area following mastectomy. There is a small fat line between the intrathoracic part of the tumour and the pericardium, thus a gross tumorous infiltration of the pericardium is improbable

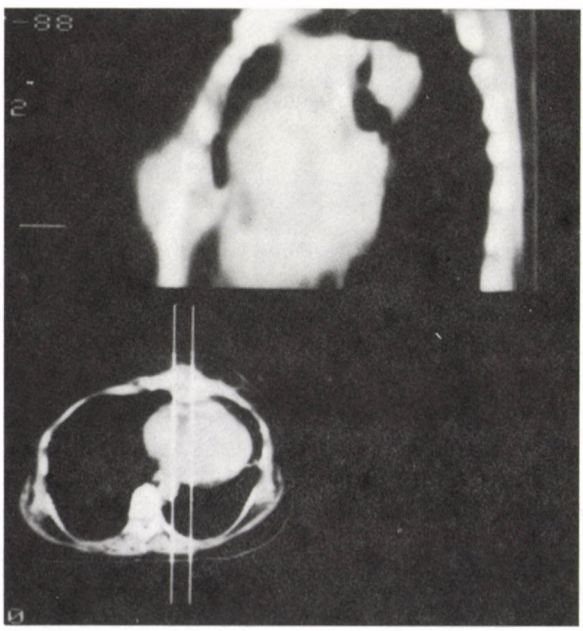

Fig. 4b. The reconstruction in the sagittal plane demonstrates the size and the shape of the tumour more accurately

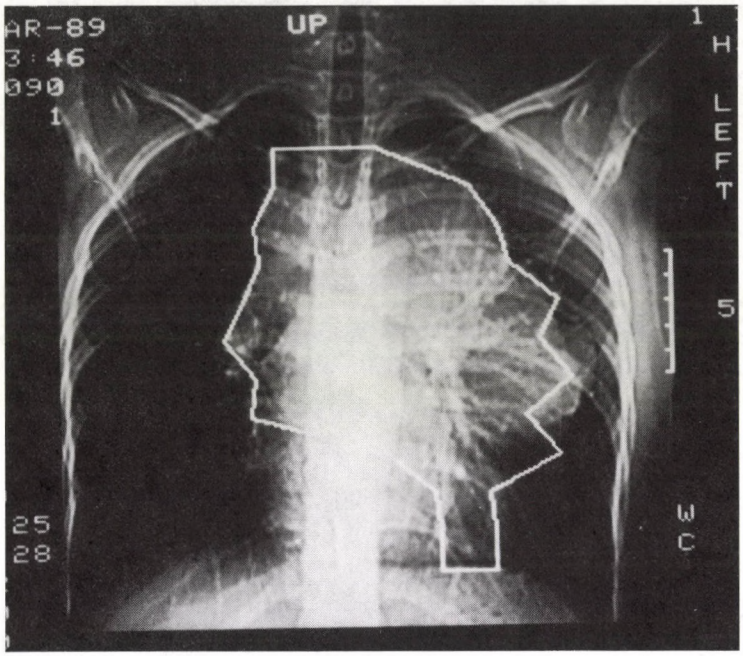

Fig. 5. The size of the tumour as projected to the topogram

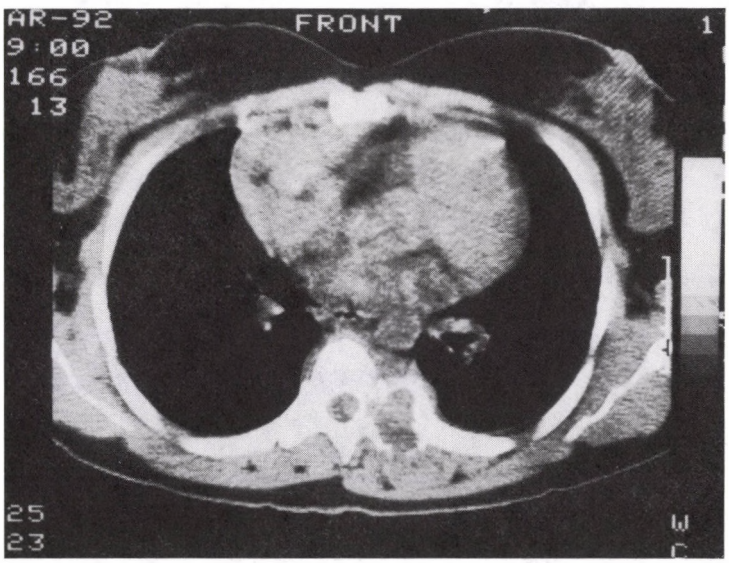

Fig. 6. The large mediastinal tumour caused by Hodgkin's disease was well seen on conventional X-ray examination. CT discovered surprisingly the lytic destruction of the costotransversal process of the VIth thoracic vertebra with an adjacent part of the rib

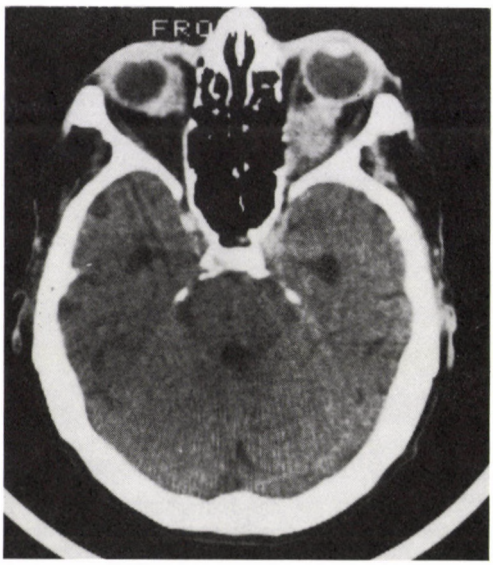

Fig. 7a. CT scan demonstrates bilateral orbital involvement caused by non-Hodgkin lymphoma. On the right side the tumour is engulfing the optical nerve, on the left there is an infiltration lying medially to the globe

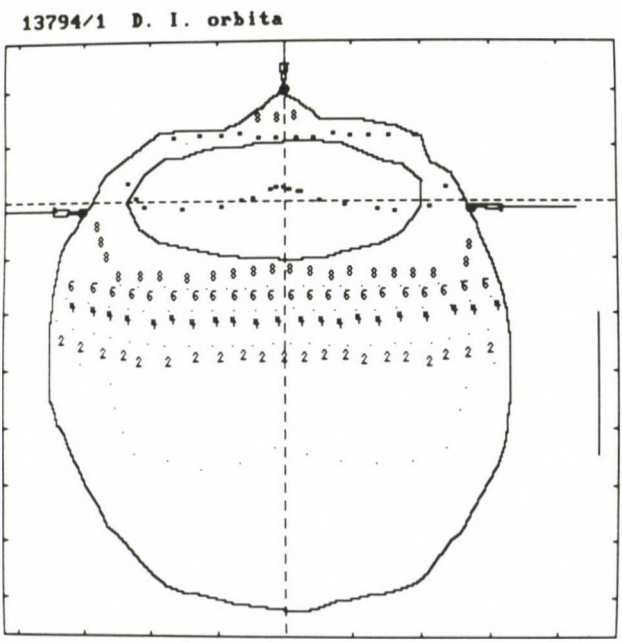

Fig. 7b. The dose distribution in the target volume seems to be appropriate by using 3 fields

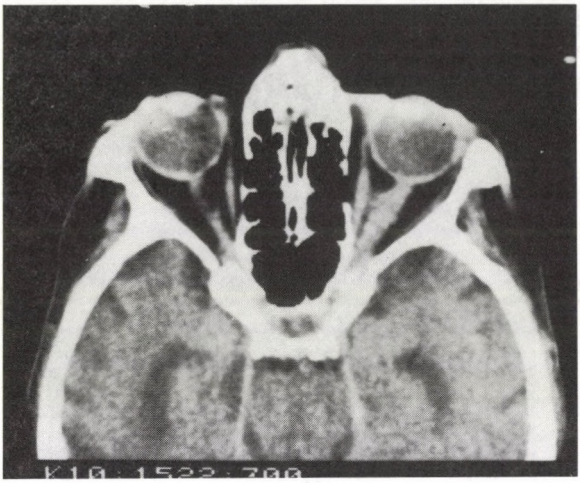

Fig. 7c. Two years following radiotherapy the regression of both tumours is complete, only minimal scar tissue is seen around the right optic nerve

(Fig. 7a-b-c). The CT scans provide information not only for treatment planning, but also in determining whether the tumour is suitable for radiotherapy. Necrotic areas in the tumour are well seen as hypodensities on the scans. If the volume of these areas is extensive, the tumour is mostly not suitable for radiation therapy as a first step and other methods of therapy might first be applied. If this form of therapy is of success, radiotherapy may be indicated later.

The CT made irradiation possible even in locations in which radiotherapy had mostly been avoided because the protection of neighbouring, by conventional imaging methods invisible organs could not be accurately carried out.

It is questionable whether it is indicated, or is necessary, to perform CT examination when the aim of radiotherapy is simply palliation. In our opinion, this should always be individually decided; however, when bone metastases are treated, CT can provide important information about the real size and local extension of bone involvement. In our experience the metastasis demonstrated by a CT scan is always larger than that shown on conventional X-ray films.

Summarizing of the above-mentioned facts and experiences makes it obvious that CT should be regarded as a routine examination in every centre of oncoradiology. Standard and appropriate application of CT is mandatory for indication of radiotherapy as well as its individual use for adequate treatment plans. Without the application of CT in the treatment planning as well as before and during radiation treatment the chances of curing the patient are significantly decreased. Proper education of oncoradiologists can only

be complete and adequate if they possess complete knowledge of how to perform CT examinations and how to read the pictures. The twin brothers of radiology: diagnostics and therapy — frequently opponents — can peacefully give hands and unite in a friendly manner in this field of discipline.

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Acta Medica Hungarica, Vol. 50/3—4, pp. 153—156 (1994)

SIGNIFICANCE OF THREE-DIMENSIONAL RADIATION TREATMENT PLANNING

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(Received: September 23, 1994)

A tumour and its environment constitute a three-dimensional (3D) phenomenon. Consequently, adequate management of the target volume (tumour + safety zone) is feasible only with a 3D treatment planning and irradiating system. The more precise planning and dose delivery involved in such a 3D treatment lead to an improved therapeutic effectiveness.

Keywords: 3D, treatment planning, dynamic treatment planning

Survey

At present threre are some 200 000 malignant tumour patient in Hungary. Some type of ionizing irradiation (gamma, photon or electron irradiation) is required during the clinical course of the disease in nearly two-thirds of the cases. Their recovery depends, among others, on the accuracy of treatment planning and on its accomplishment. Since a tumour is a three-dimensional (3D) phenomenon, adequate management of the target volume (tumour + safety zone) is feasible only with 3D treatment planning. Effective radiotherapy therefore has two prerequisites:

1. Homogeneous and complete coverage of the target volume with a given dose.

2. Maximal sparing of the tissues outside the target volume.

These criteria are met by 3D treatment planning. The first step in such treatment planning is to obtain serial computer tomographic (CT) images in parallel planes on the volume of interest. The digitalized information is then transferred directly to the treatment planning

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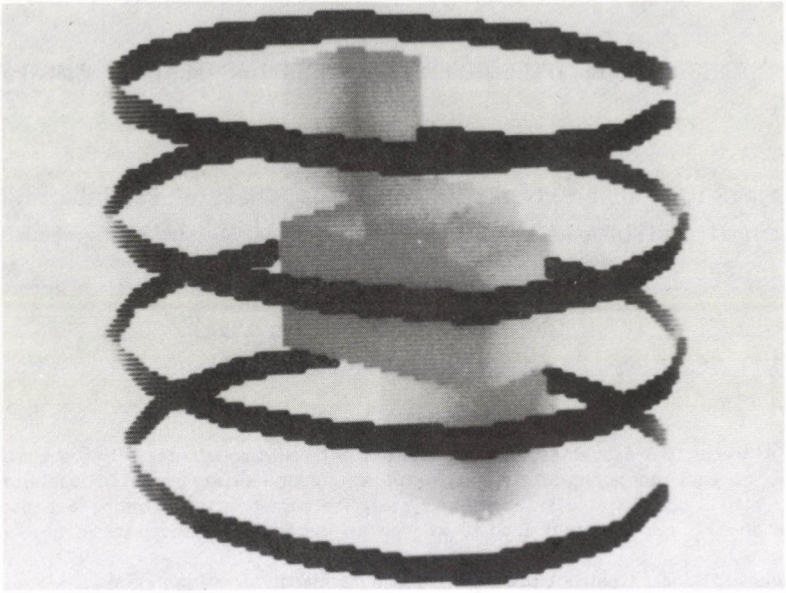

Fig. 1. Target volume (3.1 cm^3) in the midline of the brain and the relative (80%) isodose distribution of photon beams (15 MV): 8 windows were used

software /2, 4/. Following the removal of the CT data outside the body contour, 3D electron density matrices can be generated for dose calculating algorithm. The next step is 3D simulation of the radiotherapy to be delivered. Simulation is conducted according to the principle of the "beam's eye view". With this technique, the anatomic situation and radiation parameters are displayed on the screen as an eye would see them when looking out from the radiation source. In 3D treatment planning, a visual and numerical display of dose calculation is also possible.

Image evaluation is possible by displaying the relative absorbed volume dose distributions in the axial, sagittal and coronal planes and 3D perspectives (Fig. 1).

Numerical evaluation means the representation of dose distribution parameters in the system of co-ordinates, the relative dose on the abscissa and the number of volume elements or volume percentage on the ordinate.

Software developments have resulted in dynamic 3D treatment planning capable of continual adjustment of all radiation parameters. Primary and secondary collimator systems have been devised to re-shape the irradiation fields /1, 3/.

The field configuration appearing in arbitrary arrangements in the 8 windows of the multi-ccllimatorring (MCR) corresponds to the projected target volume seen from the radiation source (Fig. 2). Comparison of the

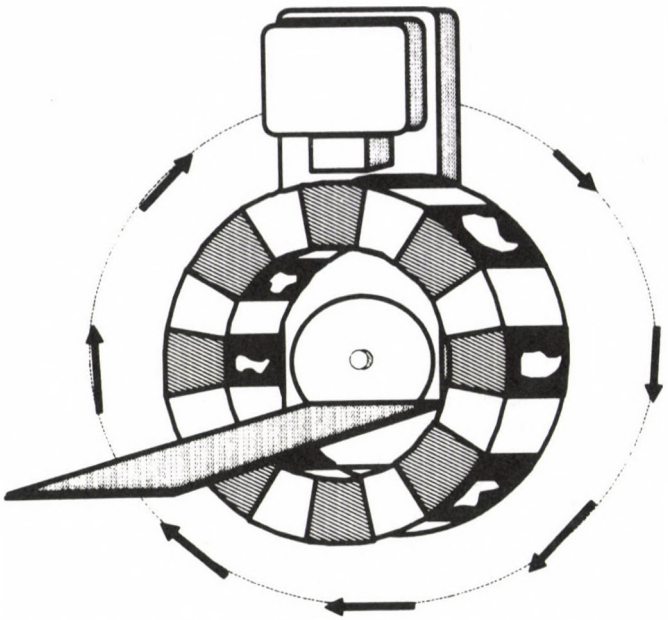

Fig. 2. Scheme of a multi-collimator-ring (MCR); overhead and operating board

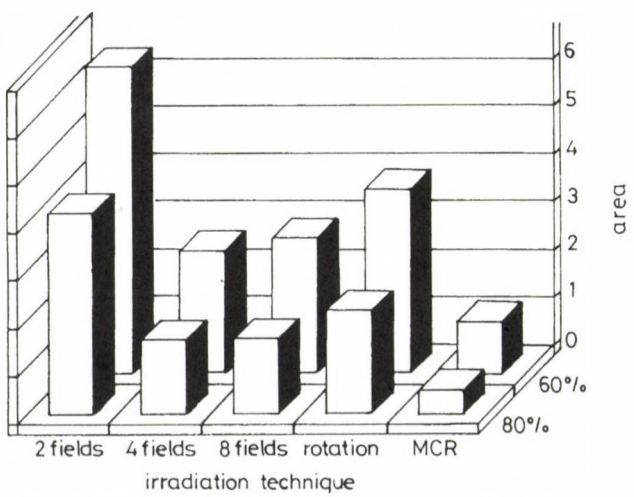

Fig. 3. Changes in target volumes within the 60 to 80% isodose range: different irradiation techniques were used

data presented in Fig. 3 clearly demonstrates that the best sparing of the intact tissues outside the target volume is feasible with irradiation through the MCR.

The introduction of 3D treatment planning in Hungary has contributed to more precise radiotherapy treatment planning and accomplishment. The improved radiotherapy reduces the incidence of recurrences, the therapy of which is more expensive than the primary treatment itself.

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Acta Medica Hungarica, Vol. 50/3—4, pp. 157—162 (1994)

THE ROLE OF BRACHYTHERAPY IN THE RADIATION TREATMENT OF MALIGNANT TUMOURS

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(Received: September 23, 1994)

The magnitude of brachytherapy doses depends on the applied dose rates: high-dose-rate or low-dose-rate techniques. Brachytherapy is usually performed as a complementary modality with megavoltage external beam therapy, but it is also used preoperatively for cancers of the uterine cervix and corpus. The most common localization is reviewed in this paper with special regard to the indications, treatment methods and prognosis of curing for rectal and breast cancer.

Keywords: Brachytherapy, breast cancer, rectal cancer, organ-conserving cancer treatment

Introduction

Brachytherapy is one of the forms of the so-called small-volume irradiation, which is done by placing applicator with nuclid source in a cavity or in the interstitium. Depending on whether the radiation source is applied manually, or through afterloading into the applicator, we distinguish the manual and the afterloading techniques forms of brachytherapy whithin the latter's computer-guided form. Depending on the applied dose rates, three forms are being used, such as: low-dose-rate, medium-dose-rate and high-dose-rate forms and their more advanced form is the pulsed-dose-rate form which may be used with low or high activating radiation source. Technical development is not a criterion in using the different activities. The formerly used low-dose-rate (LDR) technics have been replaced by the high-dose-rate (HDR) one in the meantime, but, due to our recent knowledge in radia-

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Abbreviations: low-dose-rate: LDR, high-dose-rate: HDR

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tion biology — depending on tumour cell kinetics or the gradings, either of the other brachytherapic forms is being preferred /12, 13/.

Advantages of brachytherapy can be summarized as follows:

1. Locally high dose, within fairly short time;
2. Due to a rapid dose-fall, the surrounding tissues will not be exposed to radiation.

The main disadvantages of brachytherapy are as follows:

1. It always means local irradiation therapy, in preoperative—postoperative, primary, perioperative forms;
2. It has not curative effect when applied for regional metastasis;
3. There is inhomogenity within the so-called radiation therapeutic target volume;
4. The radiation-biological effect of LDR and HDR are different.

In primary irradiation in case of cervical and endometrial tumours we apply the HDR brachytherapy afterloading technics fractionated of 30-50 Gy (Dose-unit) to the total dose of adequate dose-depths, which is completed percutaneously by the so-called megavolt therapy for regional supply /7, 10/. After an organ-preserving operation, in case of breast tumour, the LDR brachytherapy is completed by percutaneous megavolt therapy in a so-called "boost" form.

This is a similar procedure as the brachytherapeutic method applied in case of prostate tumours, where the so-called permanent brachytherapy could be done with ^{125}I nuclid seeds, which represent the local total dose in the LDR form, and similarly to the above-mentioned breast tumour, the megavolt therapy's boost form with HDR technics is being generally applied with ^{192}Ir nuclid /5, 11, 14/.

The special form of brachytherapy is an intraoperative tube-guided radiotherapy, of which quite good palliative success is being noticed, especially in the small pelvis relapses and in the regional metastases /3, 4/.

Special attention should be paid to palliative brachytherapy with the HDR afterloading technics /2, 4/ in case of mainly endoluminally progredient inoperable obstructive tumours of esophagus and bronchus.

Methods

In the Oncoradiological Center working at the "Uzsoki" Hospital, we have achieved an important experience with the curative-palliative form of brachytherapy in gynaecological carcinomas. Our results have already been published /6, 9/. Reductive-radical surgery — in oncological means: it is radical —, and could be called as "minimal surgery", which is completed by radiation therapy with percutan megavolt therapy, furthermore with brachytherapy.

We have satisfactory experience of brachytherapy technic when applying the early stage tumours of the rectum's middle or lower third and tumours of outer-upper breast quadrant. It will be below the brief summary of the therapy results of the applied irradiation technics and indications of the two localizations.

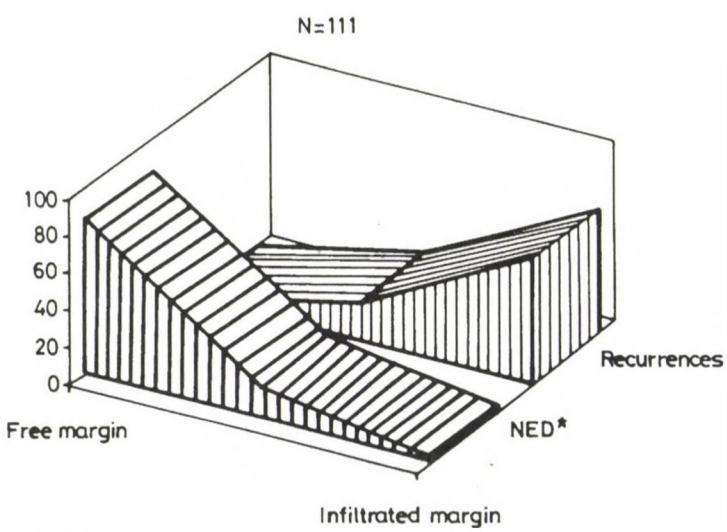
Results

I. Breast tumours

Indication: possibly in the outer-upper breast quadrant situated solitary tumour carcinoma intraductale or carcinoma ductale invasivum histology, not larger than 20 mm (T_{1a}-T_{1b}) regionally negative, or with lymph node metastasis — reaching maximum 2/10: (N_{1a}); the surgical radicality is confirmed in 2 cm to all directions, and endolymphatic spreading cannot be shown.

The indication is completed by predictive factors, such as NK activity EIC ·(extensive intraductale in situ component) hormone receptor status, etc. which is important when considering whether to apply brachy-teletherapy or perhaps chemotherapy.

In our practice, two forms of brachytherapy are being applied at present postoperatively:



*No evidence of disease

Fig. 1. Recurrence analysis of 111 women with breast tumour, with quandrantectomy and brachytherapy, based on the condition of the operation-margin

1. LDR brachytherapy of tumour-bed, manually with 50-60 Gy total dose, or perhaps the regions' megavolt therapy.
2. Tumour-bed-whole breast initial megavolt therapy with 50 Gy dose, and the tumour-bed boost interstitial HDR afterloading technics with 10 Gy dose, or perhaps by megavolt therapy of the region.

Between January, 1987 and December, 1992 we applied brachytherapy in 111 patients with breast tumour. We analyzed the recurrences form many aspects. We present our results through the side-excision — free of tumour.

II. Rectal tumours

Indications: application into the rectum's middle or lower-third, the size of the tumour does not reach one quarter of the rectum's inner circumference and its depth of expansion is till Dukes B_1 differentiated adenocarcinoma, excluding distant metastasis.

Patient-criteria should be done after a through examination, which means that besides the traditional examination, an intraluminal sonography and CT check are done.

In our present practice — possibily within four weeks after the primary tumour local excision — the following are applied:

1. Tumour bed HDR afterloading — with weekly breaks — with 6 x 5 Gy total dose.
2. Additional megavolt therapy, calculated for the rectum's region up to 24 Gy total dose.

Between January, 1990 and December, 1992 we completed brachy/megavolt therapy on 20 rectum lower third carcinomas which meant ^{60}Co teletherapy within our present number of patients.

Our therapy results are shown in Fig. 2.

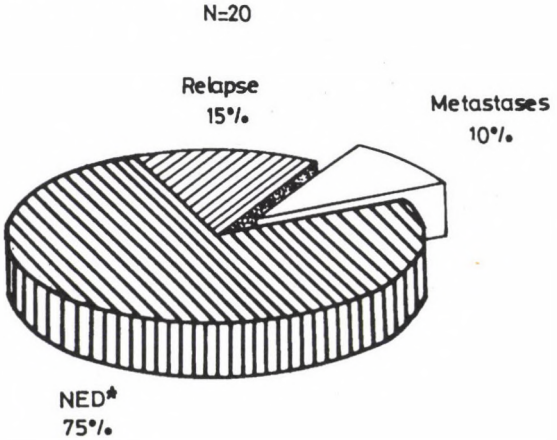


Fig. 2. Actual survival prognosis of 20 rectum lower-third patients after local excision and brachy/megavolt therapy

Discussion and Conclusions

Brachytherapy in solid tumour's radiation therapy was first introduced in the 1930s. Because of the technical conditions of that times, these were applied in a manual form, consequently, the rates of radiation exposures of the staff were high. The afterloading technique put a stop to the staff's radiation exposure and in the computer-technique an outstanding method in radiation protection has been invented. With the assistance of these monitoring equipments (CT—UH), we are able to reach the optimal dose-distribution and to minimalize the risk of the surrounding organs' radiation injury. For this purpose we are in the possession of specially developed applicators and monitoring dosimeters for irradiation. Brachytherapy is one of the used forms of the so-called "small volume" irradiation. To have a reliable clinical routine-work, the co-operation of the physician and radiation-physicist, and sometimes co-operation with a radiation biologist, may also be essential. These conditions may assure curative and palliative treatment of the tumour-type and its spreading.

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Acta Medica Hungarica, Vol. 50/3—4, pp. 163—168 (1994)

THERAPIES AVAILABLE FOR HEAD AND NECK TUMOURS

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(Received: September 23, 1994)

Treatment modalities of head and neck tumours such as cancers of the skin, lips, oral cavity, sinuses, epi-, meso- and hypopharynx, larynx and cervical soft tissues are discussed. Cervical cysts, thyroid tumours and salivary gland alterations are also examined from the therapeutic point of view. Therapeutic advices are given for the treatment of regional metastases.

Keywords: Head and neck tumours, cervical cysts, salivary gland metastasis, soft tissue tumours, thyroid tumours

Introduction

In this paper therapies available for tumours of the skin, lips, oral cavity, nasal and paranasal sinuses, epi-, meso- and hypopharynx, larynx, cervical soft tissue (cysts included), thyroid, salivary glands and their regional metastases are dealt with.

Our patients are usually sent to us from different outpatient departments of dermatology, ophthalmology, dental surgery, oto-rhino-laryngology or by general practitioners. Very often they are admitted in advanced clinical stage due either to their too late first appearance or to an inadequate attitude of the first examining physician, who may happen to belittle the patient's symptoms.

Early diagnosis, however, is of vital importance for the patient. We profess and avow the principle that tumour diseases can be cured provided the patient appears in early clinical stage, diagnosis is established within a reasonably short time and the best complex therapy currently available is given.

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Efforts have to be made to exploit the entire storehouse of diagnostic facilities, such as direct and indirect examinations, endoscopy, X-ray, US, CT, MR, aspiration cytology and angiography. These are helpful in detecting primary tumours and regional metastases.

Last but not least, comes histology to identify the very type of the tumour. Treatment modalities that may be surgery, radio- or chemotherapy or their combination, have to be evaluated in view of the precise diagnosis. In case of surgical therapy the operative technique may be classical or cryotherapy as it is with the T_1 lip cancers. With tumours of the larynx and sinuses endoscopic surgery is performed.

Laser surgical intervention with an endoscope or an external manual laser scalpel may also be justified. With tumours of the head and neck we usually apply CO_2 laser but fiberoptic Nd LAG laser may also be considered.

Since 1980, the American Coherent-type CO_2 laser equipment has been available for us. Since then, we have performed a multitude of endo- and extralaryngeal operations with high success rate for early squamous cell carcinomas.

In order to provide an acceptable quality of life to patients who have undergone major resections we have to perform reconstructive surgeries as well. For this purpose, we can use local, quilted split-skin grafts, vascular pedicled myocutaneous composite flaps, free tissue transfers and semithick flaps as well as prostheses (e.g. mandibular reconstruction) and endoprostheses (e.g. tracheomalacia) and composite grafts adapted with microvascular anastomosis (e.g. grafts with incorporated fibula for reconstruction of the mandible).

A rich storehouse of treatment modalities is at our disposal. Care must be taken to choose the most expedient one that would provide the fastest recovery and would not cover an eventual residual tumour. Similarly, the histologic examination of the "intact margins" must not be skipped.

In the following, the issue of radicality versus preservation of function is discussed.

Results and Discussion

We have to make it clear that the preservation of function may not happen to the detriment of ablation. Forced partial resections may increase the number of early recurrences that may become fatal.

Conservative surgeries require an early diagnosis since certain factors such as tumour size and localization (e.g. within the larynx) have a basic effect on the accomplishment of function saving operations.

The prerequisite of early diagnosis is screening. Screening is to be performed by the most experienced physicians who work circumspectly and with due consideration of all factors. It happens pretty often that patients must be examined in anaesthesia. Anyway, the quality of screening should be preferred to the compliance of patients. This is the only way to discover early carcinoma.

Regular ups control examination are essential even after effective treatments.

As long as the aetiology of a given tumour is not clear, the patient who has undergone effective treatment for a verified tumour has a higher probability to develop a second tumour than another without previous tumour. This explains the importance of control examinations as preventive measures for a second tumour.

In recent years we have diagnosed more and more synchronous tumours. This makes comprehensive prediagnostic examinations necessary.

Skin tumours in the head and neck region also belong to our field of activities. Unfortunately, many patients are sent to us as having a "simple basalioma". It is true that basalioma is a basal cell carcinoma but it may metastasize to the regional cervical lymph nodes and then it is by far not a "simple basalioma".

Naturally, other histological types of skin carcinomas may also occur in this anatomical region. They have to be removed by radical surgery with "intact margins" and followed by reconstructive surgery. There are many patients whose surgery was not radical enough and local recurrences became hidden by flaps of reconstructive surgery. In such a situation the patient's fate has been sealed.

Removal of skin alterations with intact margins is of vital importance in cases of malignant melanoma, which require a complex therapy. Surgical removal is feasible but consultation with a dermatologist before surgery is highly recommended.

Lip cancer is a frequent disease in recent times. In spite of being a clearly visible alteration to both the patient and the physician, patients often present with very neglected lip cancer.

Therapy is surgery, but no quadrate or wedge resection is allowed. If needed, plastic surgery of the angle of the mouth or, with extended tumours, cosmetic reconstruction is to be performed.

Cancers of the oral cavity also belong to our field. Depending on size, the lingual tumours are removed either by surgical diathermia or laser. In the presence of metastasis monoblock dissection, i.e. removal of the primary tumour and cervical lymph nodes is to be carried out. The same is valid for tumours of the floor of the mouth, the mandible and the tonsillar-lingual region. In cases of major surgical defects plastic reconstruction is to be performed. In such situations we use PM (pectoralis major) or other flaps like microvascular myocutaneous ones. For the reconstruction of the mandible, patients are managed using microvascular free tissue transfer with incorporated fibula.

The so-called "sandwich therapy", i.e. preoperative irradiation, surgery and postoperative irradiation, is still popular with tumours of the sinuses. This may involve the partial or complete removal of the maxilla with enucleation, if necessary.

We must not forget about the various endoscopic techniques, even if they have but diagnostic power with these tumours. Combination of laser with endoscopy seems to be a promising therapeutic means.

The salivary glands are also to be mentioned in this context. We have to discriminate major and minor salivary glands and their malignant and benign alterations.

Biopsy from the parotid gland is a malpractice. We can perform only aspiration cytology or surgery if lobectomy or complete parotidectomy is feasible. In fact, the histology of the intraoperative frozen sections determines the degree of radicality.

We think that the trunk of the n. facialis is always to be prepared along its branches in order to avoid the injury of the nerve. During the removal of a submandibular glandule the r. mandibularis is to be saved. In case of malignant tumour of the salivary gland block dissection is to be considered.

Epipharyngeal tumours; out of the malignant forms the transition cell and the squamous cell carcinoma are the most frequent variants. In cases of transitional cell carcinoma either with or without regional cervical metastases the therapy is irradiation at curative dosage. With squamous cell carcinoma, however, the primary tumour is to be managed with radiotherapy and the regional metastases have to be removed with radical block dissection. Unfortunately, it may happen that epipharyngeal tumours are detected in a retrograde way, i.e. first a cervical lymph node is removed and its histology makes the examiner search for the primary tumour. This order of interventions is fatal for the patient's life.

In case of juvenile angiofibroma radical surgery with preoperative embolization is the therapy of choice.

More than 90% of the mesopharyngeal tumours are carcinomas. Depending on the extension and localization of the lesion they are managed either by surgery or radiotherapy or by their combination. Radical surgeries have to be followed by reconstructive surgery to restore swallowing. Myocutaneous PM and muscle flaps and their different variants are used for this purpose.

Among the hypopharyngeal tumours, the T_1-stage ones require radiotherapy. Unfortunately, tumours of this size are but seldom detected because at this stage symptoms are minimal and patients are seldom seen by a doctor.

The T_2-T_4 tumours are to be treated by surgery complemented with radical cervical block dissection, if needed, and by postoperative irradiation. Some of the hypopharyngeal tumours which metastasize into the paratracheal lymph nodes, too, are hardly accessible for surgery or for radiotherapy radical enough. Consequently, survival rate of patients with such hypopharyngeal tumours is very poor, a 5-year survival rate occurs in 19-22%.

Laryngeal carcinomas. The T_1 carcinomas can be treated with microsurgery and CO_2 laser, radiotherapy and classical chordectomy from external exposure. The T_2 tumours, be either of supraglottic, glottic, or subglottic origin, can be removed partially or, in case of wider extension, with laryngectomy complemented with block dissection in the presence of cervical metastasis according to the "monoblock" principle.

Partial resection requires early diagnosis. As a rule, patients present with a painless swelling on one sides of the neck or both: the swelling keeps growing. In this case we have to search for the primary tumour from the epipharynx to the hypopharynx. If the findings are negative, the examinations have to be extended to the lung, stomach and kidneys, using US, CT, thyroid scintigraphy and simultaneous aspiration cytology. When all these examinations happen to yield negative results, we are allowed to perform cervical exploration complemented with radical block dissection if the intraoperative frozen sections of the lymph nodes are positive.

It may happen that an epipharygeal tumour is detected at a later time. The therapy is then irradiation. In this case, the order of interventions is not to the disadvantage of the patient, on the contrary, it is to his benefit. The removal of a single lymph node, however, would be detrimental because it would make future block dissection useless.

Most of the thyroid carcinomas represent surgical indications. We discriminate relative indications (e.g. nodular goiter and tracheomalacia)

and absolute ones (e.g., suspect malignancy). Thyroidectomy and other surgeries may injure the n. recurrens. In such cases the glottis has to be dilated surgically. Depending on the histology of the surgical preparation, surgery is complemented with radio-, cryo- and isotope therapy. The primary treatment of regional cervical metastases is block dissection that may be preventive, either elective or conservative, if a chain of non-palpable lymph nodes is removed from the clinically negative neck leaving other structures, such as the v. jugularis interna and the m. sternocleidomastoideus, intact.

When accomplishing radical block dissection we extirpate the lymph nodes on the fascia colli profunda from the supraclavicular fossa to the submandibular region on one side or both of the neck, including the m. sternocleidomastoideus and the v. jugularis interna. This operation may be carried out according to the "monoblock" principle or as a secondary block dissection.

After bilateral one-stage block dissection close observation of the patient is necessary throughout the postoperative period.

Our operations are conducted in intratracheal narcosis with intubation either through the tracheotomic orifice or through the mouth. Anaesthesiologists have a great share in the success of our work during the whole pre-, intra- and postoperative periods. Without them and their most valuable, unselfish work we were not able to perform these operations.

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Acta Medica Hungarica, Vol. 50/3—4, pp. 169—173 (1994)

THE NEUROSURGICAL ASPECTS IN THE TREATMENT OF CEREBRAL TUMOURS

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(Received: September 23, 1994)

Experience with more than 500 tumour cases operated in one year in the National Institute of Neurosurgery and the relevant oncological literature point to an important role of neurosurgery in the treatment of cerebral tumours. After reviewing the dramatic advances of neuroimaging and neurosurgical methods the main problems of neurooncology will be brought to light and the new directions of brain tumour research will be shown.

Keywords: Neurosurgery, neurooncology, cerebral tumour pathomechanism

Introduction

Brain tumours represent a small but very specific part of oncology. Their problems correspond to the large subject of general oncology. Even the fact that the tumour localizes in highly organized tissue, in the brain, which is found in a closed bony space and is submitted to the function of a blood-brain barrier and to a complicated, poorly understood autoregulation mechanism raises new aspects for consideration.

The treatment of cerebral tumours is multidisciplined. In this aspect, neurosurgeons have to take most but not all responsibility for this and this is a highly important task for them. For this purpose, tasks, opportunities and some of the related problems are explored in this paper.

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Materials and Methods

In the National Institute of Neurosurgery there were more than 500 cerebral tumour cases were operated in one year. In 1992, 553 cerebral tumours were treated surgically; 246 cases of these proved to be histologically benign, 229 cases malignant and 78 cases were transitional.

Results and Discussion

The way in which cerebral tumours arise is not known. The role of genetics is clear in Recklinghausen's and Lindau's disease; their autosomic dominant or dominant type has been verified. In experimental trials cerebral tumours have been triggered with exogenous effects or intravenously administered chemical agents (i.e. ethyl nitrosourea) /8/ and viral infection /1/. In the origin of human brain tumours the clinical investigations have failed to demonstrate any regional, dietetic or other exogenous factor /10/. However, there are reports about meningiomas developing after irradiation. The pathology of cerebral tumours is to clarified.

Dramatic advances in cell biology and molecular genetics, emerging from utilization of recombinant deoxyribonucleic acid (DNA) technologies, have provided truly novel opportunities for characterization of malignancy. We have recognized that cancer arises as a series of molecular alterations that converge to give the cell malignant properties. Whilst many more important details still need to be elucidation, it is possible, for the first time in the history of cancer research to identify specific molecular changes that account for identifiable cellular behaviors. In the last several decades, genes contributing to the development of pathogenesis have been identified. Widely investigated structural alteration and disturbances in the regulation of genes encoding growth factors and their receptors are clearly important in that they closely relate to in appropriate cellular proliferation, the pathologic feature of brain tumours /3/.

Modern neuroimaging (CT, MR) allows an early detection of the intracranial space occupying lesions in the living subject before the true symptoms appear. Apart from the early detection, these new methods make possible an exact localization of the tumour and the relationship between the neighbouring structures and the tumour. So the management of the patient is more definite because the intracranial conditions are well known. The cisterns, sulci, gyri, size and shifting of the ventricles can be seen. Whilst the development of the neuroradiological tools simultaneously decreases the invasiveness so we are getting more sophisticated information. The MR angiography and the 3D technique enables us to understand the nature of the pathological course and their relationship to the intact structures.

The new diagnostic procedure, PET, serves besides, for determination of the anatomical features, for revealing details about their functional state and metabolism.

In addition to the neuroradiology, the electrophysical (evoked potential, mapping) and laboratory (tumour markers) methods allow us to determine

(i) the biological characteristics of the tumours
(ii) their secondary change
(iii) the optimal management of the patient /6/
(iv) possible complications of the neurosurgical intervention
(v) outcome

Not only the biological features of the tumour but also their localization is an important factor of the outcome.

Widely used CT guided sterotactic machinery allows us to obtain a biopsy without the risk of an operation. There is not only the possibility of differential diagnosis but also the method has a great therapeutic value for puncturing the abscess and cysts and for brachytherapy. Deep localized tumours and angiomas have been targeted radiologically with success /4, 9/. Such an intervention combined endoscopy should be able to remove some small intraventricular tumours or to fenestrate the ventricles.

Principally benign tumours should be treated surgically. The development of surgical procedure made the challenge operating, without damaging the surrounding structure as any minor complication could have fatal outcome. In the last two decades the surgical technique has presented and excellent advance. Using mobile surgical microscopes we are able to remove all tumours in really unique localization. The areas not approached before the microscope era are operated via tiny approaches e.g. transnasally, orally. Using laser, CUSA, for removal of the hard substance lesions might be easier without pulling important structures. Intraoperative sonography makes a right placed incision possible /5/. With the help of the intraoperative evoked potential this controll functional charge preventing further complication.

The modern neuroimaging brings to light many new problems. Whilst a check-up for other complaints often diagnoses benign asymptotic cerebral lesions. The necessity of the surgical intervention has always been a hard question. The removal of brain tumours nowadays has remained a dangerous intervention, and the risk of malignancy and intratumoral haemorrhage is high. A rigorous formula cannot be ruled out. The general condition of the patient, his expected social survival, neurosurgeon's experience and his attitude are the main aspects of the decision.

The benign tumours can be operated radically. The removal of the axial and cranial base tumours is complicated because of the possible closed situation to important large vessels and cranial nerves at the base. The extension of the removal should be decided during the surgical intervention. Nevertheless, the completeness improves the clinical outcome. On the other hand, the reoperating of subtotal removed tumours might be more difficult because of adherence. The optimal strategy must be found to solve these two problems. So far the greatest neurosurgeons are not able to formulate an all-powerful rule of the management. They always mention difficulties in reaching a decision. Noticeable the direction of the discussed points relates to a radical operation providing the social outcome.

In spite of this the malignant cerebral tumours have remained incurable. There are not only surgical, but also radio-immuno-chemotherapical methods. The authors agree about the priority of surgical intervention in required localization. At the same time they disagree about the extension of the removal. Some of them /12, 13/ think the malignant astrocytomas in 90% of cases are local process so the extended resection improves the survival (five years' survival 4.7%). The investigations /7/ have demonstrated positive correlation between the less residual tumour mass and the survival long. Other investigators consider glioblastoma to be a diffuse, infiltrating tumour. Therefore, they only do biopsy and/or reduce the tumour mass. It has been shown that the tumour cells have migrated along of the white matter tracts, penetrating blood vessels, neurones, ependyma or through CSF pathways by the time of diagnosis /2, 11/.

This two-sided view is right because the malignant glioma is considered as a two compartment disease: a local tumour mass with densely packed tumour cells with broken-down blood-brain barrier and a diffuse disease composed of tumour cells located within distant normal brain parenchyma hiding behind an intact blood-brain barrier. Therapeutic strategies should continue to focus on both sides. The neurooncological research is trying to confirm these two mechanisms. How they influence each other on the molecular level? The process of invasion and its influencing must be decided.

Also the clinical experiences correspond to the above mentioned. After the more extended removal administered radio- and/or chemotherapy results in the longest survival.

The development of the surgical methods advances the more radical neurosurgical intervention. With the aim of the intraoperative techniques and the introduced real-time controlling methods we will be able to remove more extended malignant cerebral tumours.

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Acta Medica Hungarica, Vol. 50/3—4, pp. 175—183 (1994)

CURRENT PRINCIPLES IN THE SURGICAL TREATMENT OF LUNG CANCER

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(Received: September 23, 1994)

The success of surgery performed for pulmonary carcinoma is based on the selection of the patients for operation. Cytological or histological verification of the tumour prior to surgery is important as concerns the choice of the type of surgery and the complex antitumour therapy. It is currently considered that patients with tumours in stages I, II and IIIa are suitable for surgery. Operations are also performed in cases involving solitary cerebral metastases, and centrally-lying tumours which reach the bifurcation carina or the lower portion of the trachea (T_4) are similary rarely resectable.

The basic operation for pulmonary carcinoma is lobectomy. In selected cases of squamous cell carcinomas in stage T_1N_0, atypical wedge resection too may be considered. Extended surgery is also performed, depending on the size of the tumour. For all types of tumours, it is essential to take a sample from the lymph nodes for accurate staging. Prospective randomized clinical trials on 288 patients undergoing resection for pulmonary cancer revealed that extended medistinal lymphadenectomy improved the 5-year survival rate in cases of adenocarcinomas and squamous cell carcinoma, involving lymph node metastases. Intraoperative cytological examinations or frozen sections are extremely important as concerns the indication of extended mediastinal lymphadenectomy and adjuvant antitumour treatment.

Keywords: Patient selection, staging, lung resection, lymphadenectomie, complex treatment

Survey

The success of operations performed for lung cancer depends considerably on the selection of the patients for surgery. This selection involves three main aspects:

1. the histological or cytological verification of the tumour,
2. the exact knowledge of the anatomical extent of the tumour, that is the preoperative staging, and
3. the physiological status of the patient, that is the assessment of the suitability for surgery /1/.

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Table I

Place of surgery in the treatment of lung cancer as a function of the different stages

		T	N	M	Consider surgery?
Occult carcinoma		T_x	N_0	M_0	Yes
Stage I		T_1	N_0	M_0	Yes
		T_2	N_0	M_0	Yes
Stage II		T_1	N_1	M_0	Yes
		T_2	N_1	M_0	Yes
Stage III	A	T_3	N_0	M_0	Yes
		T_3	N_1	M_0	Yes
		T_{1-3}	N_2	M_0	Yes
	B	T_{1-4}	N_3	M_0	No
		T_4	N_{1-3}	M_0	No
Stage IV		T_{1-4}	N_{1-3}	M_1	No

The preoperative knowledge of the histological structure of the tumour is absolutely justified in patients who are to undergo surgery for lung cancer. It is extremely important to know preoperatively whether small cell or non small cell carcinoma is involved. However, the statistics from Hungarian thoracic surgery centres indicate that the type of tumour is known in only 50-80% of patients undergoing surgery for carcinoma.

Up to the mid-1980's, there was a wide debate as concerns the staging of lung cancer. In 1986, Mountain reported a staging system which was accepted by the Union International Contre Cancer and which has become general among thoracic surgeons since 1987 /10/.

The essence of this system is that there is no lymph node metastasis in the first stage. The T_1 tumours belonging here are smaller than 3 cm and do not reach the visceral pleura. The T_2 tumours similarly in stage I only reach the visceral pleura and at most cause segment atelectasia; they approach to within 2 cm of the bifurcation. Stage II is essentially characterized by metastases of these same tumours in the lobes and the hilar lymph nodes.

The third stage is divided into two groups. The T_3 tumour appears in stage III/A; this reaches the wall of the thorax or approaches the bifurcation and infiltrates certain structures of the mediastinum. Another important criterion of stage III/A is the N_2 metastasis, that is in the mediastinal lymph nodes. Since 40-60% of the operated lung cancer patients belong in this group, I shall return to a detailed discussion of this stage /9/.

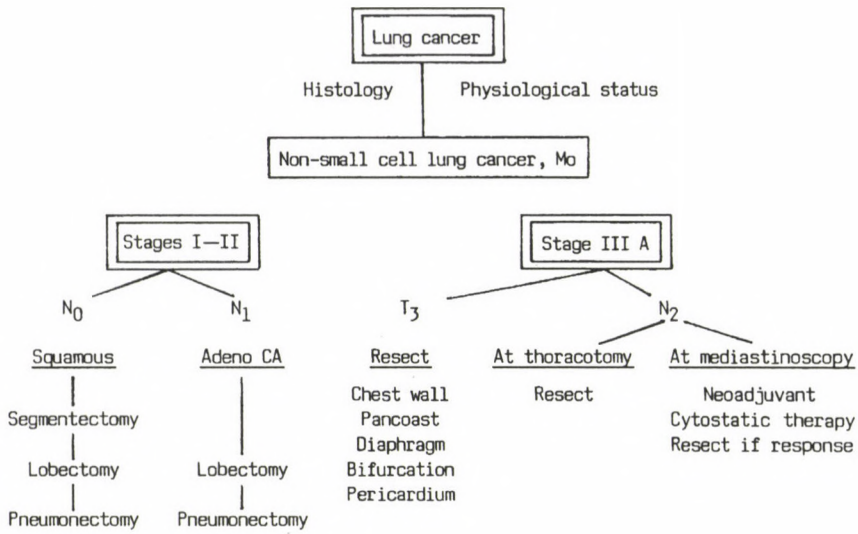
In stage III/B, the T_4 tumour extends to the arch of the aorta or to the common pulmonary trunk or to the atrial wall, and hence means technical inoperability. In the same group, N_3 implies that the contralateral or supraclavicular lymph nodes are affected, that is a situation of oncological inoperability exists.

In stage IV, the distant M_1 metastases clearly make the lung cancer inoperable.

The use of lymph node maps is of vital importance for an accurate intraoperative staging classification. The system most frequently applied, that proposed by Naruke, groups the

Table II

Course of treatment of lung cancer. I



N_2 lymph nodes from 1 to 9, and the N_1 chain from 10 to 13 /11/. The location of the lymph node metastases is not only of prognostic significance, but is also an important guide to the complex anti-lung cancer therapy /12/.

The surgical solutions of lung cancer is a function of the different stages.

Segmentectomy can be carried out in stage I, and lobectomy may be said to be a standard operation. Recently, in place of segmentectomy, we perform atypical mechanical wedge resections with staplers in high-risk, mainly elderly patients and especially in cases of squamous cell carcinomas. These operations must definitely be avoided in cases of adenocarcinomas, because of the high rate of local recurrence. In general, segmentectomy or mechanical wedge resection is employed in 1-5% of resections performed because of lung cancer. With more extensive tumours, we carry out bilobectomy or pulmonectomy. Sleeve resections have become generally accepted during the past ten years, and we have used this technique increasingly more frequently in lung cancer patients. 80-90% of the sleeve resections are carried out because of lung cancer and their number has quadrupled in the past seven years /6/. The Hungarian thoracic surgeons Ungar and Keszler earned wide-ranging international recognition for their good results with parenchyma-sparing sleeve resections /3, 15/.

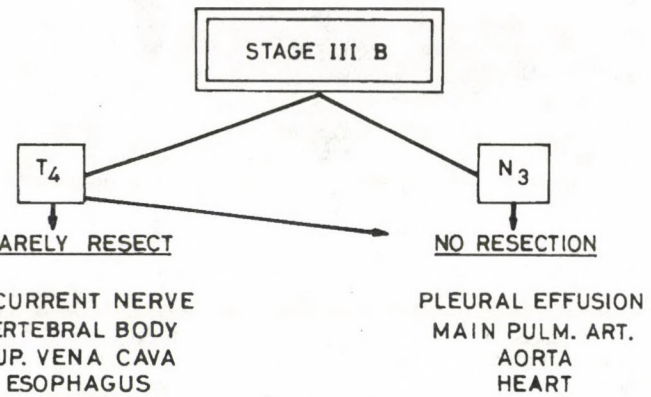


Fig. 1. Course of treatment of lung cancer. II

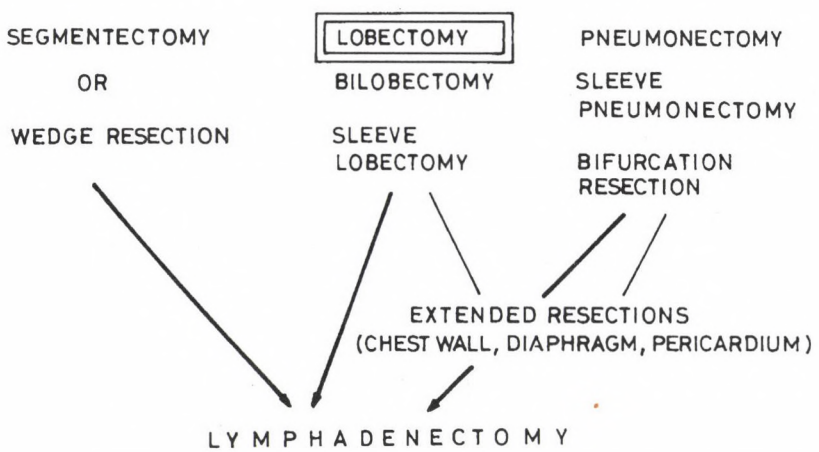


Fig. 2. Types of operations performed for lung cancer

In cases of T_3 tumours or Pancoast tumours, we perform resection of the wall or of the apex of the thorax, respectively. With centrally located tumours, in many cases we provide the vessels intrapericardially, and in fact resection of part of the left atrium may also occur. In the event of tumours infiltrating the trachea bifurcation, we carry out some type of bifurcation resection or sleeve pulmonectomy.

An interesting change may be observed as concerns the surgical conception for N_2 tumours. If the preoperative staging suggests that the superior mediastinal lymph nodes are affected, we always employ mediastinoscopy. In the event of a positive biopsy, combined neoadjuvant cytostatic treatment

			5-year survival	Total
Stage I	T_1N_0		83%	72%
	T_2N_0		65%	
Stage II	T_1N_1		56%	49%
	T_2N_1		48%	
Stage III A	T_3N_0	Chest wall	56%	
	T_3N_0	Proximity to carina	36%	19-23%
	$T_{1-3}N_2$		20%	

Fig. 3. Five-year survival rates of patients resected because of non-small cell carcinoma of the lung as a function of the different stages

is begun and, if the lesion exhibits a regression, resection surgery is performed /8/.

In stage III/B, resection can be carried out only very rarely in cases of phrenic paralysis. With Pancoast tumours or tumours causing cava superior syndrome, palliative surgery comes into consideration to alleviate the complaints. In cases of distant metastases, only a solitary cerebral metastasis comprises an exception to the inoperability /5/.

A feature clearly associated with surgery for lung cancer is lymphadenectomy.

The survival rate is best in cases of T_1 epithelial carcinomas in stage I, where more than 80% of the patients survive for five years. With the appearance of the N_1 lymph nodes in stage II, the rate falls to 40%. Only 10-15% of the patients are in this stage.

In stage III/A, the five-year survival rate is on average about 20%, or somewhat lower, It is noteworthy that in this stage a survival rate of 50% is achieved if tumours that reach the wall of the thorax but do not give lymph node metastases are removed together with the wall fo the thorax /2, 13/. The survival rate is also better in cases of tumours where bifurcation resection is performed and there are no lymph node metastases. Here the five-year survival rate is above 30%.

For a given lymph node status, one factor influencing the survival rate is the size of the tumour. Thus, for N_2 cases the five-year survival rate is 30% for T_1, but only 12% for T_3 /6/.

A further such factor is whether there were metastases in only one lymph node or in several, and whether these occurred at one or more levels, and whether the metastasis penetrated through the lymph node wall.

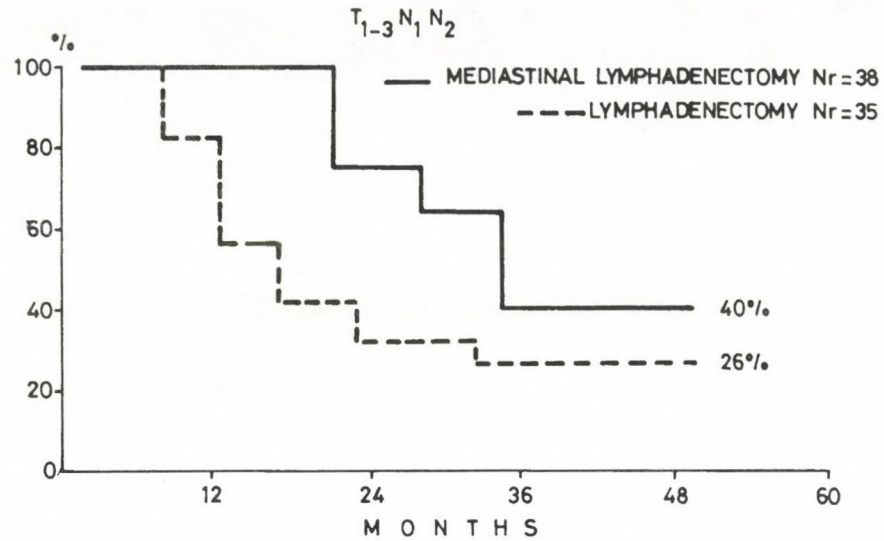


Fig. 4. Survival of squamous cell carcinoma cases (U.I.C.C. 1987 stages)

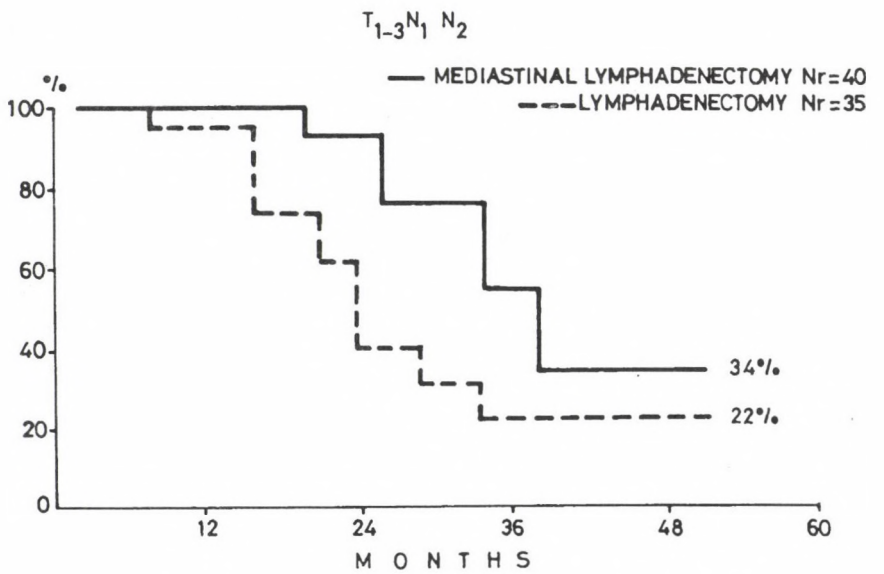


Fig. 5. Survival of adenocarcinoma cases (U.I.C.C. 1987 stages)

An improvement of the survival rate in these cases is to be expected only from complex anti-tumour treatment. The prospective randomized study by Martini revealed that the survival rate of patients with adenocarcinomas and large cell carcinomas was significantly improved by postoperative irradiation of the mediastinum and subsequent combined cytostatic treatment /7/.

A few words about lymphadenectomy and extended mediastinal lymphadenectomy. Watanabe processed the data on 2000 lung cancer patients and concluded a fairly large proportion of such tumours do not metastasize in accordance with the regional lymph process. Additionally, jumping or skipping metastases too may be observed, when a lymph node step or level is omitted from the metastasization. On this basis, he recommended systematic exstirpation of the mediastinal lymph tissue in all lung cancer cases /16/. Indeed, in a paper published one year later, following total exstirpation of the mediastinal lymph nodes in cases of N_2 epithelial carcinomas originating from the left superior lobe, Watanabe also removed the contralateral lymph nodes via a median sternotomy /17/.

To demonstrate the place of extended mediastinal lymphadenectomy, I should like to give an account of our own randomized patient material. During 288 lung resections which we carried out because of non-small cell pulmonary carcinoma in a three-year period, extended mediastinal lymphadenectomy was performed in every second case. In the traditionally operated group, only the regional lymph nodes were removed, or the lymph node chain around the main bronchi and those lymph nodes that were suggested by palpation to be enlarged or pathological. The surgical mortality in the traditional group was 1.62%, while that in the extended mediastinal lymphadenectomy group was 2.45%. Perioperative complications occurred in greater number in the extended group, and the average amount of blood used was about double in this group. The extended mediastinal lymphadenectomy increased the duration of the operation by 40-50 minutes. The five-year survival rates in the two groups were 42% and 38%, in favour of extended mediastinal lymphadenectomy. The difference was not significant.

When the data were further broken down according to histological type, and the lymph node-positive cases were examined separately, analysis of the two Kaplan–Meyer curves for the lymph node-positive squamous cell carcinomas indicated a significant result in favour of extended mediastinal lymphadenectomy, with rates of 40% and 26%.

A study of the survival rate in cases of adenocarcinomas giving lymph node metastases also revealed a significant difference in favour of mediastinal lymphadenectomy, with figures of 34% and 22%.

In consequence of these data, it has become our practice in recent years to follow the decision concerning resectability by removal of the lymph nodes from the upper mediastinum, from the bifurcation, from beside

the main brochi and from the pulmonary ligament. If the intraoperative cytology or a frozen section shows a metastasis in any lymph node, then extended medistinal lymphadenectomy is performed /14/.

The place of surgery in the treatment of pulmonary carcinoma is clear-cut. The results cannot be improved, or only partially, by the surgical radicality. A factor that decisively influences the survival rate is better patient selection.

This begins with the preoperative staging, continues with the intraoperative cytology or frozen sections, and is completed with the detailed postoperative histological examination. The intraoperative staging is extremely important as concerns the selection of the type of operation or of lymphadenectomy, and also determines the modifications of the complex antitumour therapy.

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Acta Medica Hungarica, Vol. 50/3—4, pp. 185—193 (1994)

CHEMOTHERAPY OF ADVANCED BREAST CANCER

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(Received: September 23, 1994)

The treatment of clinically overt metastatic breast cancer, despite several treatment modalities (biological response modifiers, megatherapy with autologous bone marrow transplantation, growth factors, new agents, etc.) is in a static phase. In the decision-making one has to consider the patient's age, her menstrual state, the metastatic site, previous adjuvant and/or postoperative treatment modalities. Roughly there are two treatment forms, the hormonal and the cytostatic ones. Endocrine therapy should be given as follows: 1. only for low risk group, 2. gestagen or antiestrogen therapy is the choice for the first step, 3. if there is a progression in 3 months, the hormonal treatment should be changed to cytostatic combination, 4. if there is a progression beyond 3 months further hormonal therapy can be considered. The efficacy of endocrine therapy is 30%. In patients with advanced breast cancer chemotherapy provides a response rate of 30 to 60%, however total survival of the patients does not improve substantially. Doxorubicin containing regimens are more effective, however no response in total survival can be obtained. New plant alkaloids and altered treatment forms will probably influence survival. Taking all these into consideration one has to decide on the quality of life of the breast cancer patients.

Keywords: Advanced breast cancer, decision-making, hormonal treatment, chemotherapy, quality of life

Introduction

Both the incidence and mortality of breast cancer, are increasing in industrialized countries /13/. The Hungarian statistical data are shown in Tables I—II—III. It is supposed from the occurrence rate that the disease

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Abbreviations: ER = estrogen receptor, PGR = progesteron receptor, CPA = Cyclophosphamide, Mitox. = Mitonzantron, MMC = Mitomycin C, ADM = Adriamycin, MTX = Metothrexate, 5-FU = 5-Fluorouracyl, DDP = cis-Platin, IFO = Ifosfamide, CMF = Cyclophosphamide + Metothrexate + Fluorouracyl, FAC = Fluorouracyl + Adriamycin + Cyclophosphamide, AC = Adriamycin + + Cyclophosphamide, MMM = Mitozantron + Metothrexate + Mitomycin C, VACMFP = Vincristin + Adriamycin + Cyclophosphamide + Metothrexate + Fluorouracil + Prednisolon, MVAC = Metothrexate + Vincristin + Adriamycin + Cyclophosphamide, VCFP = Vincristin + Cyclophosphamide + Fluorouracyl + + Prednisolon, AV = Adriamycin + Vincristin

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Table I

Mortality data

Year	Total	Breast
1980	27937	1800
1985	28893	1990
1990	31221	2097
1992	32676	2232

Table II

Effectiveness of cytostatic drugs in advanced breast cancer

Name of the drug	Response (%)	Survival (mos.)
CPA	33	12
MITOX.	35	14
MMC	28	10
ADM	38	15
MTX	28	11
5-FU	27	10
DDP	20	15
IFO	20	17

Table III

Combined chemotherapy of advanced breast cancer

Name of the drug	Response rate (%)	Response duration (months)
CMF	48	11
FAC	60	21
AC	44	24
MMM	51	16
VACMFP	46	19
MVAC	57	17
VCFP	51	17
AV	58	12

will be presumably observed in one case of 10 to 12 newborn females. Despite sophisticated adjuvant chemotherapy trials in patients without clinically recognisable metastases, in three quarters of the patients overt metastases will develop in 2 to 10 years and the patient dies in a rather short period /3/.

The treatment of clinically overt metastatic breast cancer is in a static phase and with rare exceptions, the disease is considered fatal in outcome /18/. Despite innumerable modalities (using hormonal agents, cytostatic drugs, high dose chemotherapy with autologous bone marrow transplantation, biological response modifiers, etc.) the cure rate has not changed substantially, however, the disease free survival, and, which is more important, the quality of life of the patients has improved dramatically.

In treating patients with metastatic breast cancer it is important to consider the following components: the patient's age, her menstrual state, the metastatic site, previous adjuvant and/or postoperative treatment modalities. Roughly there are two subgroups in the treatment forms, the hormonal and the cytostatic treatments.

Survey

Endocrine therapy

Manipulation of the endocrine system has been the choice of treatment for several years in premenopausal women with metastatic disease. Decreasing the level of endogenous estrogen performed either by surgical ocophorectomy or with ovarian irradiation has led to a therapeutic result of approximately 30% /8/. The discovery of steroid receptors contributed to the selection of patients for endocrine treatment. The objective response rate is 50 to 60% in patients of estrogen positive (ER +) tumours and this rate further increases by 10% if the progesteron receptor (PGR) is also positive. Tumours/metastases having ER values less than 3-10 fmol/mg respond to endocrine treatment in less than 20% compared with neoplasia of 70% response rate with more than 30 fmol/mg of protein. The PGR level seems to be even more important than ER positivity /6, 21/.

Surgical ocophorectomy in menstruating women with ER + tumours causes an objective response of more than 30% lasting for approximately 8 months. Radiation castration with 2000 cGy shows the same effect, however its result appears 8 to 10 weeks later /5/.

As patients with ER-tumours respond to these procedures rarely, such interventions are not indicated in this group. The considerable exception in the ER-group is: patient more than 35 years of age, slow growing metastases and long disease-free interval.

Other endocrine manipulations as adrenalectomy or hypophysectomy are doubted in the presence of novel medicaments as aminoglutethimide or LHRH analogues /16/.

Antiestrogens

The nonsteroidal antiestrogen tamoxifen has become the most widely used hormonal therapy for patients with metastatic breast cancer and it seems to be as active as the operative procedure. It exerts its main antiproliferative effect by competing with estrogen for binding to ER proteins. Besides, it also alters the mechanism of signal transduction and leads to a cell-cycle blockade /26/. In postmenopausal women tamoxifen decreases the normally elevated gonadotropin level and it influences the previous steady state of hormone balance.

Tamoxifen is currently recommended for postmenopausal patients with advanced disease in a daily dose of 20 mg. (Doses exceeding this level do not show better results, however the toxicity is more prominent.) The drug is also active in ER + premenopausal women, however its value is less well documented /19/. Among others Buchanan et al. /5/ compared tamoxifen with surgical oophorrectomy in 1986. The median response duration and the overall median survival failed to achieve statistical significance. However, the side effects were more pronounced in the oophorectomized group. Considering this the castration is not recommended in the premenopausal group.

The effectiveness of tamoxifen is around 30% in postmenopausal women with metastatic cancer. The median time till progression is about 5.4 months /6/.

The toxicity of tamoxifen is minimal and transient /22/. Weight gain due to fluid retention, vaginal bleeding caused by hormonal inbalance, edema, skin rash, nausea and vomiting, leukopenia and thrombophlebitis, abnormal liver function appears in less than 3% of patients. Hypercalcaemia and tumour flare occur in about 5% of the treated patients.

New analogues like droloxifene, toremifene, zindoxifene and trioxifene do not show better results than the parent compound.

Progestogens

Medroxiprogesterone acetate (Provera, Upjohn, Farlutal, Pharmacia) and megestrol acetate (Megace, Bristol Myers—Squibb) are available. The antineoplastic effect of these drugs is poorly understood. They may cause antitumour effect mediated by hormone receptors. On the other hand, they influence the hypothalamic — pituitary — adreanal axis with resultin insuppression of adrenal steroid production /7/.

The most frequently given dosage is 500 to 1000 mg per os once a day for the first month, then twice a week until progression.

The drug is almost exclusively administered in postmenopausal women. Its effectiveness is about 30% /11/. However, it should be noted that the greater the PR content the higher the response rate /2/. The response lasts about 4 months. The results are better in hormone naive patients. In case of liver involvement the effectiveness is uncertain. There is no clear cut evidence that the combination with Tamoxifen shows better results /10/, however, it can be stressed that the results are probably better if the hormonal treatment had been begun with progestogens instead of Tamoxifen /6/.

The side effect of the treatment includes facies lunaris, fine tremors and leg cramps, weight gain, fluid retention, hypertension, etc. in 10 to 50% of the cases. Worsening of diabetes and hypercalcaemia should also be stressed /27/.

Aminoglutethimide and aromatase inhibitors

The major sources of the estrogen protecting breast cancer cells are the ovaries, pituitary, fat and the adrenals. The incidence peak of breast cancer is in the 7th decade, where the overwhelming production of estrogen is localised to the adrenals. The normal cells of the gland produce androstenedione. The latter is converted by an aromatase reaction in peripheral tissues to estrone and estradiol.

Aminoglutethimide blocks several cleavage enzymes and it is capable of inhibiting the conversion of cholesterol to pregnenolone, blocking all adrenal steroid synthesis. Therefore, patients treated with aminoglutethimide are usually given glucocorticoids to prevent Addisonian side effects /15, 23/.

The treatment with aminoglutethimide is as effective as other hormonal treatment forms and the former can replace the surgical intervention in postmenopausal women.

The routine dosage is 2 x 250 mg Orimeten (Ciba) twice daily combined with 40 mg/day hydrocortisone for several months.

The overall response rate is 35% in previously untreated postmenopausal patients. The median duration of the response is about 5 months; it should be stressed that in previously Tamoxifen treated patients the response rate is 25%. It is important to mention that the drug is more likely to induce objective response in sites of osteolytic metastases.

Most of the side effects can be observed in the first 6 weeks. They consist of skin rash, which disappears spontaneously during the treatment and doses not need the cessation of the medication, ataxia, dizziness and lethargy, and hypertension /12/.

Luteinizing hormone releasing hormone analogues

These agonists, including buserelin, goserelin, leuprolide decrease FSH and LH secretion and are recommended for premenopausal breast cancer

patients with intact ovarian function. The response rate is 30 to 40% for previously untreated women. Interestingly, there are some data which show the effectiveness of the drug also in postmenopausal cases /17/.

Amenorrhoa persists as long as the patient is on treatment.

Side effects are minimal and they consist mainly of hot flushes which do not need to stop medication.

Combined endocrine therapy

Combining several hormones does not seem to be superior to single hormonal therapy.

Guidelines for endocrine therapy

Regarding survival, breast cancer patients can be divided into two groups, a low-risk and high-risk groups. Patients above 35 years with high ER and PR content those, with monolocalised bone or pleura or soft tissue metastases and those in whom metastases appears as late as beyond 2 years, are assigned to the low risk group. In premenopausal patients with low ER and/or PR content, in those with one or more visceral metastases and where the metastases appearing within 2 years after the diagnosis the endocrine treatment is ineffective: all these patients are in the high risk group.

The best results of hormonal treatment can be seen in soft tissue metastases followed by pleura and bone metastases. Practically no response can be seen in visceral lesions and there is a risk of worsening in liver metastases treated with hormones.

Regarding these observations the recommendation of hormonal therapy is as follows:

1. hormonal treatment only for low risk patients is recommended,

2. gestagen or antiestrogen therapy is the choice for the first step of hormonal therapy,

3. if there is a progression in 3 months, the hormonal treatment should be changed to cytostatic drug combination,

4. if there is a progression beyond 3 months of hormonal treatment, further hormonal therapy can be continued.

Chemotherapy

In patients with advanced breast cancer chemotherapy evokes a response rate of 30 to 60%, however long-term remissions or cure occur rarely /1/.

Taking this into consideration, the aim of the medical oncologist often remains improvement of quality of life instead of healing of the patient. The increasing enthusiasm regarding the success of adjuvant therapy has led to force cytostatic treatment even in advanced cases.

Many drugs are effective in the treatment of breast cancer. The medicaments most commonly used are listed in Table II. However, current trials indicate that polychemotherapy is superior to monotherapy regimens in disease-free survival. It is also known, that doxorubicin-containing protocols yield a 10 to 20% higher response rate than polydrug regimens without doxorubicin. Unfortunately, improvement in general survival has not been demonstrated. The same applies to CMFVP when compared with CMF. Vincristin and prednisolon does not improve survival, but these drugs provoke more side effects. The currently used polychemotherapy protocols are listed in Table III. The differences came from the patient selection, tumour localisation, stage, etc. The response rate is higher among patient who present with disease predominantly in soft tissues than those with bone or visceral metastases. On the other hand, one should not be neglect the contribution of local treatment is certain situations. If there appears for example a local relapse 4 years after the diagnosis has been made, the cytostatic treatment is not indicated, but it can be effectively treated with local excision and irradiation when restaging excludes distant metastasis /15/.

The optimal duration of the treatment is contraversial. Current trials indicate that a continuous use of chemotherapy results in a twofold longer disease-free interval than polychemotherapy in six courses, however no difference can be observed in total survival time /24/. As there is no clear cut evidence on the superiority of either form, the medical oncologist has to focus on the quality of life instead of causing more side effects. Comparing doxorubicin containing regimens to mitoxantrone polychemotherapy, it can be concluded that the antitumour effect is the same, however the toxic effects are reduced in the latter form of treatment /20/.

Another important question is combination of polychemotherapy with endocrine treatment. Theoretically, the tumour may contain several clones which are differently sensitive to altered manipulations. Unfortunately, there is no evidence that combining the two modalities results in better survival.

Recommendations for chemotherapy in advanced breast cancer patients

High risk group patients should be treated with multidrug chemotherapy. It is necessary to consider the type of adjuvant treatment. If it has been made by using CMF, an anthracyclin derived combination should be considered either with or without hormonal treatment for a minimum period of 6 months.

If progression appears the formerly used polychemotherapy should be stopped and replaced with another regimen. The best results can be reached

by using Vinblastine + Mytomycin C in previously anthracyclin treated patients.

New drugs and treatments

The taxus brevifolia derived taxol is the most promising new drug in the treatment of advanced breast cancer be it previously treated or not. The recommended starting dose is 225 mg/m^2 every 3 weeks /4/. The 30 to 50% response rate seems to be of great importance in previously treated patients. Side effects like neutropenia, asymptomatic tachycardia, myalgias, alopecia, vomiting, diarrhoea, etc. should be considered.

Other plant-derived drugs like topostatin and podophyllotoxins are also of scientific importance, however no randomized studies are available todate. Bone marrow transplantation, haematopoietic growth factors are also promising in the treatment of generalised breast cancer. The former modality makes megatherapy possible and prevents death from myelosuppression /28/. The latter allows us to alter the protocols using 4 week courses. Unfortunately, it is not unanimously confirmed, that the 2 weeks cycle is superior to the classical 4 week courses /25/.

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Acta Medica Hungarica, Vol. 50/3—4, pp. 195—203 (1994)

NEW TRENDS IN THE SURGERY OF GYNAECOLOGICAL TUMOURS

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Operative treatment of gynaecological tumours includes exploration, staging and removal of tumour for both therapeutic and diagnostic purposes for advanced processes the optimum conditions of postoperative supplementary treatment are to be developed. In contrast with the earlier view suggesting that a malignant tumour of a given organ is always the same in every case, nowadays tumour heterogeneity is emphasized. Multicentrical co-operative surgical research should widen our knowledge. Well-designed and well-equipped centres should be made available for our patients suffering from gynaecological malignancies; gynaecological oncology needs well-planned functioning special clinics/wards.

Keywords: Surgical treatment, gynaecologic malignancy, cancer cervical, endometrial, ovarian, vulvar

Introduction

Our aim in the surgery of gynaecological tumours is to develop — in accordance with modern principles of surgery — interventions more and more radical in respect of curing of the affected organ; the organ left behind after operation should contain healthy and active tissue as much as possible. For this purpose, the tumour should be removed completely with growing accuracy.

The prognosis of a patient with malignant tumour depends on the stage of malignancy and on the therapy in use /15/. The modern tumour surgery adjusts to the stage of malignancy as far as possible. In prognostication of benign cases, the intervention should not be radical; the affected organ should keep most of its functions. In malignant cases on the other hand the operative area should be extended, i.e. the operation should be more radical.

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The therapy is the more successful the better the prognostic factors are known. Therefore, it is important to establish the risk groups with patients who — because of having malignant tumour — need increased screening tests and care /12/.

The FIGO Stage Arrangement (1988), which was built up in the last one or two decade's experiences, is also important /8/. Accordingly, in the judgement of gynaecological tumours, against all other criteria we should decisively rely on histopathological findings, as clinical details may not agree with the result of the diagnostic examination.

The high numbers of therapeutical failures refer to the fact that gynaecological oncology is still in progress. Failure is often caused by a wrong judgement — that was based — before therapy on the size and extension of the tumour /17/. To develop a right opinion about the success of the therapy is strongly influenced by certain tumour-specific properties. The general stage of the patient's health, his state of nourishment and his current immune status affect the success/failure of the therapy.

There is no doubt that the rapid development of screening, diagnostics and therapy has not overshadowed operative gynaecological approach; instead, it becomes more and more indispensable. (Staging operations, radical operations, etc.) Removal of the tumour tissue, and also of potential tumour tissues by excision has remained the key of therapy. Moreover, it should be noted that there exists no virtuous surgical solution that cannot be completed by radio- and/or chemotherapy in a favourable manner. So we do not specify the exclusiveness of the surgical approach, instead we specify its place in the therapy arsenal. That is the principle that should lead us gynaecological oncologists — in every case, even when we establish the therapy plan.

The surgery of cervical cancer

In curing of intraepithelial carcinoma, instead of using the earlier generally accepted cone biopsy, some authors suggest colposcopically aimed punch biopsy, an intervention that they consider less aggressive than cone biopsy is. In this respect, however the literature is inconsistent; we unambiguously prefer cone biopsy. Of course, in the judgement, favourable or unfavourable experiences in using conization play a significant role. We are sure that our own operative technique is not "aggressive". By the way, a barely or less "agressive" intervention has to be combined with a closer and

more accurate follow-up method. The aimed biopsy's concept does not reckon with the possibility of an intracervical process and deliberately or not deliberately, does not think of the therapeutical and preventive importance of conization /6/.

The prerequisite of a correct judgement a right operative approach of cervical cancer, is a clear survey of the possible ways of the metastasis-forms of the tumour. As it is known the tumour firstly infiltrates the cervix and it islater that it breaks into the lymphatic vessels. It is yet unknown how deep the stroma invasion must be to cause such break in. Several observations whos that even a minimal stroma invasion can cause spreading into lymphatic vessels. From that moment on, the risk of extracervical spreading is high. The results of morphometrical measuring refer to the fact, that there is a connection between tumour size and the incidence of lymph node metastasis /12/.

Furthermore, we should not leave out of consideration that the tumour can break into blood-vessels: a problem that was undeservedly avoided before. There are three ways for spreading in the lymphovascular system. 1. lateral infiltration through the paracervical lymphatic vessels towards the parametriums and the wall of the pelvis; 2. caudal spreading: the tumour may break into the stroma of the vagina or can even take over the place of the stroma. In case of major spreading the tumour can spread to the lower part of the vagina and to the inguinal lymph nodes; 3. cranial spreading: the cervical cancer takes over the endocervix and the lower part of the uterus. The FIGO did not take this often occurring spreading into consideration w when he developed his Stage Arrangement. This way of spreading can contain the major part of the lower part of the uterus. In case of primary irradiation, a certain part of the tumour is staying out of the isodose lines. In the cases reported by Janish and Köbli /12/ this type of cervical cancer was apparent in 30%.

The incidence and the risk of the lymph node metastasis is stage-dependent. In stage Ib it is 20%, whereas in IIIb or IVa it is already 60%. The further spreading through the para-aortical lymph node chain can spread as far as the supraclavicular region.

For a long time there was no consistent opinion about cervical microcarcinoma. The definition has changed during the past few years. According to the new arrangement to distinguish stage Ia1 from Ia2 is based on the findings that was the grade of the invasion from the base of the epithelium within 5 mm, and the horizontal spreading within 7 mm. A tumour larger than this belongs to stage Ib.

The clinician's dilemma is about the fact that the difference between Ia1 and Ia2 is based not on an exact measurement, but on the pathologist's definition. Determination of the depth of tumour invasion is important only when it exerts an influence on prognosis and therapy. Literary data and our own observations show that in stage Ia1 we can be satisfied with a simple hysterectomy, whereas in stage Ia2 we have to intervence as in stage Ib. Therefore, there is a need for pre- and postoperative irradiation and Wertheim's operation, for in case of a 3 to 5 mm infiltration the risk of metastasis is 5% /15/.

Up to stage IIb of cervical cancer Wertheim's operation has remained the standard treatment. In case of advanced processes (IIIa and IIIb) on the other hand, only radiation therapy, comes into consideration.

The operative therapy of cervical cancer has to be completed with radiotherapy. We have no final viewpoint about adjuvant and neoadjuvant chemotherapy.

The surgical solution of a recurrent cervical cancer is a major challenge for oncological gynaecologists, as the recurrencies in most cases grow slowly and appear locally. There can only be one aim at the solution of the surgery of relapses i.e. to remove the entire tumour. Therefore, in case of centrally located recurrence mainly partial or entire exenteration is the solution of choice. If the recurrences touch the wall of the pelvis and bone metastasis has developed, the prognosis does not leave much hope.

The life chance of a patient with cervical cancer depends on the tumour size, the type of histology and the lymph node metastases. An important factor is the histology of the surgically removed lymph nodes. In case of positive finding the chance for a five-year survival is about 60%, whereas in case of negative finding it is 85%.

The surgery of endometrial cancer

The combined surgery-pathological stage arrangement of endometrial carcinoma had been used until a couple of years ago /8/. The reason for changing the stage arrangement has been the recognition of several prognostic factors which decisively influence the life chance of the patient /21/. The most important changes happened in early cases, where the judgement of the myometrium infiltration played a significant role. The depth of the invasion shows a good correlation with the risk of the development of lymph node metastasis and, therefore, with the survival chances of patients with endometrial cancer /7/. The infiltration of the tumour can be well judged by a transvaginal ultrasound examination as well.

In the old times primary irradiation was the treatment of choice of uterine cancer. Nowadays, however, in most cases the patients with increased surgery risks can be operated without any particular danger. The advantage of the operative solution is that it moderates the developing of local recurrences and — by precise examination of the abdominal cavity — assures a more exact staging.

Operative solution has made it clear that in patients at stages I and II the isthmical region is affected in about 23%, the adnexal structures in 5 to 6%; the pelvical lymph nodes in

10 to 11%; the peritoneal fluid contains tumour cells in 12-13% of the cases /4/. These factors strongly influence the prognosis. Besides affection of the blood vessels, finding of the lymph node is particularly important. We have the worst prognosis when metastasis can be shown at para-aortic lymph node /14/.

Just like the infiltration depths of the tumour, the recognition of the type of histology in particular of the malignant serous and clear cell tumours, and the grade of differentiation (greading: ploidy, the rate of the S-phase) are to be specified and the receptor content is to be determined. These factors can be well examined in the uterine curette from an abrasion even at the price of repeating the curettage.

The presence of the node metastases shows a good correlation with the other prognostic factors. In case of favourable and unfavourable prognosis the presence the node metastasis is $\leq 10\%$, and 40%, respectively /7/.

Of all endometrial malignancies 2/3 are represented by low-risk carcinomas. This means carcinomas of high progesterone-receptor content, diploid chromosome composition, glandular or glandular-solid endometrioid type, which infiltrates on the endometrial surface or infiltrates the myometrium, too, maximum to its 50% (stages IA and IB). Risk for the affection of the lymph nodes is below 5%, therefore, there is no need for lymphadenectomy or postoperative irradiation. Peculiar for the high-risk tumour is, the lack of progesterone receptors, the aneuploidy, an unfavourable type of histology, the solid type of growing and a strong infiltration of the myometrium. If the carcinoma infiltrates more than 50% of the myometrium (IC stage) then it is justified to remove the lymph nodes of the pelvis and — in case of macroscopic suspicion of metastasis — to remove the para-aortic lymph nodes, too /7/.

In the "high-risk" group, the affection of the pelvic or para-aortical lymphatic node is $>5\%$.

As in the treatment of uterine cancer, the surgical solution is still at the first place /17/; in our own practice it is performed after preoperative irradiation. Looking at the recovery rate for patients at the same stage, omission of surgery acts against survival by 10 to 25%. Twice as much is the incidence of recurrence in patients with primary irradiation, than in those with primary operation /17/. Thus the two procedures should be combined, not contrasted.

In the knowledge of the risk factors, in cases of stage IA and IB, we do not have to reckon with positive lymph node findings; therefore, there is no need for Wertheim's operation; a simple hysterectomy by keeping the principle of ablasticity in view is satisfactory. (Immediately before operation we close the cervical canal and during the operation we do not grasp the corpus, but for example, we pull the uterus with stitches placed in the ligamenta rotunda, we hold the infundibulopelvic ligament with broad cuff, etc.) /15/.

The literature is inconsistent about uterus removal with vaginal cuff. Careful surgical closing of the cervical canal in case of patients operated with uterine cancer is important, as it can reduce the number of the recurrencies in the vagina stump, and there will be no need for hysterectomy to be made by cuff. Tumour spreading into the vagina, on the other hand, makes the removal of the affected parts indispensable.

Ovarian cancer

It is generally accepted that removing of the whole tumour induces a prolonged survival. If the residual tumour is small-sized, or reduced in mass, the general condition of the patient can considerably improve. The success of an operation, resulting in a residual tumour smaller than 2 cm depends on the tumour localization and the extension of the tumour into the upper abdominal area /11, 13/.

According to the generally accepted view, the life chance of a patient operated with ovarian cancer depends on the stage of the tumour, the histological differentiation and on residual tumour /18/.

The cornerstone of the treatment of ovarian cancer is tumour reduction during primary operation and staging /5/. Operative intervention with keeping of the conception is only justified in cases of young women who are at stage Ia and who still want to give birth. The prerequisite of this intervention is that the tumour is one-sided, without adhesions and rupture of the capsule, and it is histologically highly differentiated. If family planning has been finished, the leftover ovaries should be removed even by a subsequent operation for it cannot be excluded that tumour is bilateral and, if so the consequences on the leftover ovary are unestimable. It is also justified to remove the uterus, and an omentectomy is recommended.

The aim of the surgical solution of the advanced ovarian cancer is to remove the tumour as extensively as possible, for the surival time is proportionally reduced by the residual tumour quantity. Burghardt et al. /5/ recommend pelvic and para-aortical lymph node dissection as well. In spite of this removal of lymph nodes in cases of ovarian cancer cannot be made obligatory, yet it seems that if it is possible through the open abdomen (for example: the operating surgeon is — occasionally — familiar with lymphadenectomy) it can be done /2/.

The literature is inconsistent about the role of the so-called "second look" operation in the surgery of ovarian cancer. Today the second-look operation is recommended primarily for diagnostic purposes, when the aim of the success of this treatment method is the objective judgement and the planning of the further therapy cannot be reached with not invasive methods. During the second operation the supplementary tumour reduction does not necessarily add to the length of the survival time; however, there are experiences showing the contrary. We agree with the latest establishment, which suggests that the laparoscopy and the laparotomy contribute only little to the prognosis, and strongly reduces the quality of the remaining life of the patient /2/.

Tumours of low malignancy (borderline tumours) of the ovary deserve particular judgement. The literature is contradictory about the prognosis of patients with this kind of tumour. In our own experience the prognosis of the illness is good compared with the prognosis of other malignant tumours, even if transperitoneal metastasis is present.

Chemotherapy successfully completes the surgery of ovarian cancer. The treatment method which fits to the stage arrangement is followed by a treatment method that adjusts to the patient more. It is characteristic of this tendency that many — earlier thought inoperable — primary operation of elderly patients can be done with success. The individual treatment also means that the surgeries made today are more radical than the earlier ones were, without a higher surgery risk of the patients. This can happen because of the higher level of postoperative treatment, the modern anaesthesia, and, finally, the richer experience of surgeons. The technical development also supports the cytoreductive surgery treatment.

The first articles about the ultrasound surgery appeared in the second half of the '80s. The CUSA (Cavitron Ultrasonic Surgical Aspirate) has an operating head made of titanium that vibrates with 23 000 hertz fix frequency; by changing the amplitude, the high water-containing and the low collagen-containing tissues are shuttered and washed away. In our own experiences with the help of this machine it is possible to reduce ovarian tumours in size and to remove lymph nodes lying next to vessels and nerves /19/. It needs special attention that — by getting to know more and more prognostic factors — the view that takes the restriction of radicality and the keeping of the organ into consideration.

The surgery of vulvar cancer

In the diagnosis and treatment of vulvar cancer, we can witness an improvement regarding the early recognition and the chances of surviving. There are some changes in treatment introduced in the last 10 years /10/. The treatment became particularly individual, especially at the tumour's early stage.

Looking at earlier statistic data, the proportion of the incidence was about 4% for all malignant gynaecological tumours. In the last years the number of vulvar intraepithelial neoplasia (VIN) has grown, especially in young people /1/.

The reason for this is probably that the population undergo more infections with oncogenic viruses. Just like in the case of pre-invasive changes of cervical cancer, we distinguish the stages I, II or III of vulvar intraepithelial neoplasia (VIN). The basis of classification — in this case too — is the right sample and right histological processing.

Recently an individual surgery technique has been introduced into the treatment of vulvar cancer, that equally includes amended radical vulvectomy and — in case of early-staged tumours — local removal of the tumour. It

seems that the 2 cm healthy excision of the tumour impairs neither the survival, nor the risk of relapse. But even the new organ-keeping ideas do not give up lymph node dissection /16/.

By the removal of the inguinal and femoral lymph nodes the tumour size, the depth of invasion and the affection of the vessels has to be noticed. In the treatment of tumours the removal of the inguinal and femoral lymph nodes and the irradiation of the inguinal region are the most common interventions.

In our department, we have found out from the histology that the tumour can spread to the opposite side. So the lymph node dissection is essential and the postoperative irradiation has to be done in every case.

Systemic treatment of the tumour has not yet been solved. The reason for that is that we do not have enough experience about the treatment of rare tumour. Very little literature is known that would evalue methods combined with chemotherapy. So nowadays it is hard to have a final viewpoint about systemical treatment.

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Acta Medica Hungarica, Vol. 50/3—4, pp. 205—219 (1194)

SURGICAL TREATMENT OF ESOPHAGEAL AND GASTRIC CANCER

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(Received: September 23, 1994)

Based on literary data and his own practical experience the author summarizes the short history and possibilities for surgical therapy of the esophageal and gastric cancer diseases.

Keywords: Cancer, esophageal, gastric, surgery

On the surgical therapy of esophageal cancer

Malignant esophageal tumours make some 2% of the total number of cancers and 4% of the tumours of the gastrointestinal tract /15/. Its incidence is variable: in Northern China 100, in Japan 50 whereas in the so-called western countries 4 to 5 cases per a 100 000 population are revealed every year.

In Hungary, similarly to many other countries, tumours of the digestive tract belong to the rarely occurring diseases. Due to their late recognition, incurable cases are often encountered, and a considerable part of the operated cases show a rather high postoperative mortality because of this reason and due to the unfavourable survival, esophageal cancer is one of the most problematic fields of oncological surgery.

The esophageus is located in three parts of the body, viz. in the cervix in the thorax and in the abdomen. It is closely connected with organs of life importance. This is why esophageal surgery belongs to the category of major operations. The treatment of esophageal tumours may be of multidisciplinar approach, but the primary role of surgical intervention is beyond question.

It was in 1913 that Franz Torek, a surgeon of Hungarian origin, operated a 60-year-old women for tumour of the mid-third of the esophagus. He removed the tumorous esophagus, then made an esophagostomy at the neck and made a gastrostomy. He repaired the continuity of the intestinal tract by a rubber pipe /45/. The patient survived the intervention by more than 10 years and died of an intercurrent illness. The first intrathoracal replacement after removing the

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0236-5286/94/$ 4.00

esophagus was performed by Ohsawa in 1933 /32/. Thus, in 1913, and again in 1933, it was shown that esophageal carcinoma was not an incurable disease and that even a reconstruction is possible which improves the patient's postoperative quality of life.

Pastorino /35/ processed the data of 4930 esophageal resections from the literature of the years 1955 to 1985. He found that between the first and the last third of this period the mean value of postoperative mortality fell from 30% to 9% while the 5-year-survival rate rose from 8% to 19%. He found similar, but more expressed, trends in the Chinese and Japanese literature. In those countries the survival rate for unselected patients rose from 9% to 23%. This rate attained even 90% when only operations performed in an early phases of the disease were taken into account.

The results cannot yet be generalized yet. In the majority of the cases the diseases are already in advanced state when the diagnosis is established. Due to the wide extension of the tumour and/or existence of remote metastases, the disease is incurable. In advanced cases, long-term survival cannot be expected, the efforsts are mainly of palliative character and they are aimed at restoring of swallowing. This is the primary goal of treatment of patients with esophageal carcinoma, besides, prevention of complications, prolongation of life and if possible, total healing are aimed at.

Radical surgery of esophageal tumours

Analyzing the factors of postoperative survival Skinner /40/ concluded that the survival is influenced by transmural tumour extension and by lymph nodes metastases. It seems to be well-founded that the state of the lymph nodes is the most reliable prognostic factor for esophageal resection /3, 5, 20, 22/. In the surgery of esophageal tumours three, partially antagonistic, attitudes can be distinguished, we hallmark these by the names of Orringer, Skinner and Akiyama.

Orringer /33/ recommends transhiatal removal of the esophagus, without opening the thorax. In his opinion long-term survival is not influenced by the degree of lymph node dissection if so it is not justified to make efforts for removing lymph nodes that may be burdened by metastases. For this reason the surgical interventions performed as "blunt-bind" dissection are preferred by this author.

According to Skinner /38/, who makes efforts to remove the tumour radically, by en bloc dissection of the esophagus and the surrounding tissues being under favourable conditions, i.e. when preoperative staging promises curative surgery, uses this technique and thus enables to cure a part of the patients even if lymph nodes are being affected in the remaining cases local recurrence occurs most rarely if this method is applied. However, postoperative mobidity and mortality are not negligible in such cases /41/.

Akiyama /1/ performed subtotal removal of the esophagus and recommended resection of the stomach which he approached from the side of the lesser curvature, he combined this intervention with standard lymphadenectomy (Fig. 1). (According to the interpretation of the present time standard

Fig. 1. Typical lymph nodes positions around the esophagus

lymphadenectomy means a double-field lymphadenectomy which extends to the mediastinum and the upper half of the abdomen.) In Akiyama's opinion, gastric resection from the side of the lesser curvature is necessary from the oncological point of view because lymph node metastases (sometimes so-called jumping metastases) often occur in this position which in case of tumours in the middle third may even jump over intermediary lymph nodes, transferring metastases to the celiac trunk lymph nodes.

For intrathoracal carcinomas all the three methods accept the necessity of subtotal esophagectomy because of the intramural tendency of esophageal tumours and their multifocal appearance.

Replacement can be made on the site of the esophagus, i.e. intrapleurally or retrosternally, and an antethoracal replacement is also possible. It may be an advantage of the retrosternal replacement that possibly developing local recurrence cannot "catch" the organ used for reconstruction of the intestinal tract's continuity and a postoperative irradiation of the esophageal bed remains possible. Insufficience of the sutureline of cervical anastomosis is followed by a reduced risk if the replacement is retrosternal.

Wong /50/ in his operations signed the altitude of the anastomosis before examining how radical the esophageal resection from a transthoracic approach may be. He found that in an anastomosis prepared in the cervical esophagus enables the surgeon to extend resection by less than 2 cm. With postoperative randomized examinations, Chasseray /8/ showed that in case of an anastomosis prepared in the cervical esophagus that the distance between the resection line and the tumour was twice but such an extension of the operation was not followed by a reduced number of perioperative complications or by a more favourable long-term prognosis.

Out of the neoplastic diseases of the thoracic esophagus the above-mentioned Akiyama operation is performed most commonly all over the world. In these operations the lymph node dissection is pyramidal in shape, it is the most extensive in the abdomen and the lower mediastinum and the least radical in the upper mediastinum and at the cervical level. The intensive development of the surgical techniques and the high-level perioperative intensive therapy have made it possible for Akiyama et al. /4/ to recommend extended lymphadenectomy at least for selected cases.

Extended — so-called three-field — lymphadenectomy

Dissemination is much more typical of esophageal carcinoma than of gastric cancer. The stomach has a network of lymphatics around itself which may serve as a protective factor against tumour metastases. In the submucous layer of the esophagus, the lymphatics run longitudinally, therefore, e.g., in case of a middle-third tumour, tumour cells may arrive at the cervical or celiac trunk lymph nodes earlier than those running in the lymphatics through in esophageal muscular layer arrive at the lymph nodes located at their own level. When the esophagus is affected, lymph node metastases may appear far from the routine areas of surgical resections. This may be why this time more and more surgeons, mainly Japanese ones, recommend an extension of the operation, i.e. performance of a three-field lymphadenectomy.

The extended surgery is approached with thoracotomy from the right side, the fatty tissue is totally removed from the upper part of the mediastinum en bloc. The lymphnode chains have to be dissected and removed which adjacent to the recurrent nerves, to the art. bronchiocephalica, to the sup. ven. cava as well as to the right and left paratracheal region. In the neck, the supraclavicular region is approached from both sides to remove the internal jugular vein as well as the paraesophageal lymph nodes and the lymph nodes adjacent to the n. recurrens are to be removed. These considerably prolonged operations are accompanied by preoperative and postoperative complications which are definitely elevated in number. It has been reported several times that also mortality is higher. Further studies and a careful evaluation is needed until it will be clarified when and on what kind of selection is it possible to choose an operation of higher risk in the hope of prolonged survival.

In case of tumour located in the lower part of the esophagus Peracchia /36/ recommends removal of the esophagus through a right-sided thoracotomy combined with standard lymphadenectomy, he prepares the anastomosis in the thorax at a high level. Extended (three-field) lymphadenectomy seems to be indicated when tumours of high segment of the esophagus are to be removed or in selected cases when the mid-part of the esophagus is to be removed. The same author performed removal of the esophagus without thoracotomy. Such an

intervention does not make possible a correct lymph node dissection. Peracchia selects this operation only in cases in which an operation performed with thoracotomy is contraindicated.

We at our University Clinic of Surgery select the type of surgery on practically similar indications. In case of middle-third tumours, which make the majority of the cases, we choose the classic Akiyama operation as a routine procedure, the same intervention is preferred by us for so-called early carcinomas.

Palliative operations in the treatment of esophageal tumours

Many surgeons suggest that the role of the surgeon in the treatment of esophageal carcinoma is only palliative /29, 33/. Belsey /6/ is of the same opinion except that he stresses the surgeons have an active role in prolonging the patient's life and alleviating his suffering. Belsey says that such a correlation is more important than the statistical analyses. The age of life and the risk of complications cannot contraindicate operation.

In a high per cent of the patients presenting with complaints the tumour has already broken through the wall of the esophagus and has developed remote metastases. Dysphagia appears dramatically, its cause can easily be proved, however, it should be known that two thirds of the esophageal circumference has already been infiltrated before dysphagia manifests itself. Development of a tumours fistula towards the airways may be followed by rapid and catastrophal consequences for the patient /18/. The survival time of the untreated patient lasts not longer than several months. Aglutition, aspirations and the consequent conditions create intolerable circumstances for survivors.

Although patients with hopeless esophageal carcinoma have already tried to heal by other procedures (cytostatics, irradiation, laser, etc.), surgical solutions occupy the first place even in the palliative therapy /19, 46, 50/. The goal of treatment should be worthy survival, not a simple one (McKeon /28/). Resection is the best palliation not only from oncological but also from functional view.

When metastasis is to be found preoperatively in all the three regions (cervical, mediastinal and abdominal region), long-term survival cannot be expected even after complete lymph node dissection. If the tumour belongs to the highly malignant cases, long-term survival cannot be expected even if the microscopic finding is negative for metastases. In such cases, extended lymphadenectomy is not justified. There is no doubt that remote metastases and locally invasive tumours need only palliative resection, the usual type of lymph node removal is not indicated in such cases /4/. Many authors have suggested that out of the palliations the resection should be preferred when the operation is aimed at the recovery of oral nutrition /6, 10/.

If an esophageal tumour cannot be resected, especially if this condition is manifest and associates to esophago-tracheal/bronchial fistula the surgical "bypass surgery" may be the solution of choice. A fistula associating to severe dysphagia may be accompanied by continuous aspiration, thus, it may be intolerable. If the general condition of the patient is good enough to allow a major operation, restitution of ability of swallowing with bypassing of the esophagus is a right method for elimination of an imminent fistula. The stomach is used for this purpose the most favourably. If the stomach is not accessible fit for replacement e.g. in case of a previous gastric resection, an isoperistaltic colon segment can be used instead. In the absence of fistula between the esophagus and the airways, one should take care of unburdening of the remaining esophagus segment. If the tumour has caught the recurrent nerve and the patient continuously aspirates because of a defective mechanism of swallowing then even "bypass surgery" will have no role in the palliation series /2, 47/.

Part of the patients cannot be subject to palliative bypass surgery because it needs satisfactory general condition of the patient. In such cases restitution of swallowing can be achieved by insertion of an esophageal endoprothesis. In cases of tumours extending to high levels the introduced endoprothesis sometimes gets displaced and thus the proximal part of the prosthesis may slip into the pharynx. This is possible because in these cases the esophageal segment over the tumour is not long enough to push back the tube into the tumorous segment. In border cases the operation can be completed by the cervical approach. Slipping up of the prosthesis is prevented by loose narrowing thread loops immediately below the pharynx-esophagus border.

In cases in which even the introduction of the endoprothesis is impossible (tumours in a high cervical level), the catheter pharyngo-esophago-(gastro)-stoma may be indicated /48/.

The early esophageal carcinoma and the so-called Barrett malformation will be discussed in the following separately.

On the surgical treatment of early esophageal carcinoma

In the last few years, owing to the improvement of diagnostic facilities and very carefully performed studies about patients with early complaints, the number of early discovered carcinomas has considerably been in-

creased especially in Japan. At present, we have an available number of data on the characteristics of the so-called early carcinoma /11, 31/.

It is justified to divide into three subgroups the tumours qualified as T1 according to the TNM classification of 1987. These are intraepithelial (EP), mucous type (MM) and submucous type (SM) carcinomas. Lymph node metastases do not develop from EP tumours, so develop but rarely (5%) from MM tumours and develop at a high frequency (about 40%) after the changes have extended to the submucosa /11/.

The type of interventions to be selected depends on the facts, namely endoscopic mucosectomy or removal of the esophagus without thoracotomy is the intervention of choice when the tumour is of subgroup EP or MM. In case of MM carcinoma operation without thoracotomy or that with right-side thoracotomy, may be possible.

When the tumour is of the SM type, thoracotomy to be approached from the right side, together with radical lymphadenectomy, is suggested, however, there are surgeons who are satisfied with removal of the esophagus without opening the thorax /34/.

On the surgical treatment of endobrachiesophagus — Barrett's esophagus

When endobrachiesophagus is present, the distal segment of the esophagus is covered by columnar epithelium. For this reason the esophagus appears to be short from the inner side. This picture, which was first described by Norman Barrett in 1950, is thought to be an acquired phenomenon at present. To make the valuation more accurate, there is a widely accepted rule, viz. we speak of Barrett's esophagus only in cases in which it appears as an at least 3 cm long formation as a continuing of the gastric mucosa. According to Bremner's theory /7/ the site of the esophageal mucosa disappears as a result of gastroesophageal reflux and its site becomes covered by columnar epithelium, which is growing more rapidly. Skinner /39/ suggests that reflux and Barret's metaplasia are parallel phenomena, their co-existence shows a rather high correlation, however, causal relationship between the two conditions could not be established. It is of importance that malignant transformation occurs 30 to 125 times as frequently in cases of endobrachiesophagus than in the intact esophagus; the cases have been increasing in number. According to DeMeester /9/ each diagnosed case in the indicator of 20 non-diagnosed ones, furthermore, 40% of the patients have no complaints suggestive of reflux.

The surgical treatment includes treatment of the complications and prevention of malignant malformation. Although it has not unequivocally been proved, formation of dysplasia in the Barrett epithelium can be prevented by antireflux surgery or the metaplasia regresses following surgical treatment /39/.

What is to do when Barrett's anomaly has been diagnosed?

Medication should be initiated even in unintentionally recognized cases which are free from complaints. In such cases the condition should be checked at 6 to 12 month intervals. For this purpose multisite biopsy material should be taken from the metaplastic-dysplactic area in order to determine the degree of dysplasia and, if it is justified, to judge the possible progress and, thus to recognize the possibly carcinomatous character. Regular checking is justified even after antireflux surgery and the so-called brush cytological test should be performed yearly to demonstrate possibly appearing malignant cells.

The indication of the conservative surgical treatment is similar to that of the operations performed for gastroesophageal reflux, namely a reflux resisting internal treatment and/or a severe (ulcerative) nonhealing Barrett's ulcer complicated by stricture. Surgical solution may be necessary when serial aspirations occur in a condition practically free from complaints or when the patient objects long-term medication. The conservative treatment usually means some form of fundoplication, however, Fékété /14/ suggests to perform total duodenal diversion aimed at preventing regurgitation of duodenal contents into the esophagus.

Radical surgical treatment (resection) may be indicated in severe cases of dysplasia and histologically proved carcinoma in which the changes are progressing in spite of a correct conservative therapy /23, 42/. It should be noted that at present pathologists and clinicians agree well in that high-grade carcinoma and in situ carcinoma are synonymous designations, and also in that resection is the method of choice /43/.

Barrett's carcinoma has a bad prognosis and the survival shows a close correlation with tumour stage. It seems to be widely accepted that dysplasia — high-grade dysplasia — is the best indicator of malignant malformation in cases of Barrett's esophagus /25/.

The resection surgery of the esophagus is a major surgical intervention. Nevertheless, it may load to favourable results (reduced morbidity and mortality) in centers in which the team is well-trained in organizing both the preoperative and postoperative work well /30/.

On the surgical treatment of gastric cancer

Carcinoma, the gastric tumour of epithelial origin makes 95% of the tumorous diseases of the stomach. It occurs most frequently in Japan and in Chile. Its yearly incidence in these countries ranges between 7 and 10/10000 population. In the USA and in Canada the corresponding frequency ranged between 0.8 and 1.4/10 000. In Hungary, about 4000 patients died of gastric cancer yearly.

The incidence of this disease shows as downward trend all over the world. It is of interest that the localization of the primary affection is changing. In the USA two thirds of these tumours appeared in the antrum primarily and only 10% were recognized around the cardia. According to a recent study published by the American College of Surgeons (ACS) /49/ 31 and 14% occurred in the upper and middle thirds of the stomach, respectively, and the changes extended to the entire stomach in 10%, only 26% occurred in the lower third.

It is of importance that the cardia cancers are more aggressive than the tumours occurring elsewhere in the stomach, and it should be stressed that the treatment of proximal tumours is technically more complicated than the changes appearing in an antral position. The former need wider circumspection, more intense training and experience.

Gastric cancer needs surgical treatment, this principle has generally been accpeted. The recent development of anesthesia, the high-level direction of the perioperative period and the refined surgical techniques have made it possible to perform radical operations. As the type of surgery and the degree of radicality there are controversies in the world of surgeons.

The first succcessful gastric resection was performed by Theodor Billroth on 29 January, 1881. His patient survived the intervention by 14 months and died of dissemination of tumour.

In the '40s and '50s of the present century, total gastrectomy was the operation of choice in the therapy of gastric cancer /26/. This radical approach abandoned later, because it seemed to be undesirable in increase gastric cripples in number. Still later, it became apparent this condition was not a consequence of the agastric state, and it was also proved that this undoubtedly poorly tolerated, condition can be avoided on the basis of functionally applied principles.

The studies directed to the lymph circulation of the stomach were initiated early in the century /21/ (Fig. 2). Spreading of gastric tumours through the lymphatic system was first described in detail by Sunderland et al. /44/ in 1953. Radical dissection became a standard component of gastric cancer surgery soon. Gilbertsen /17/ stated in 1969 that morbidity and mortality increased and the 5 year survival was reduced after extended surgery. Subsequently, in the USA and in a great part of the Western world — except for certain centerns — the principle and practice of radical lymph dissection was suppressed wile elsewhere, first of all in Japan, it has remained an indispensable component of surgery, its gradually refining principles and techniques are being applied with favourable results.

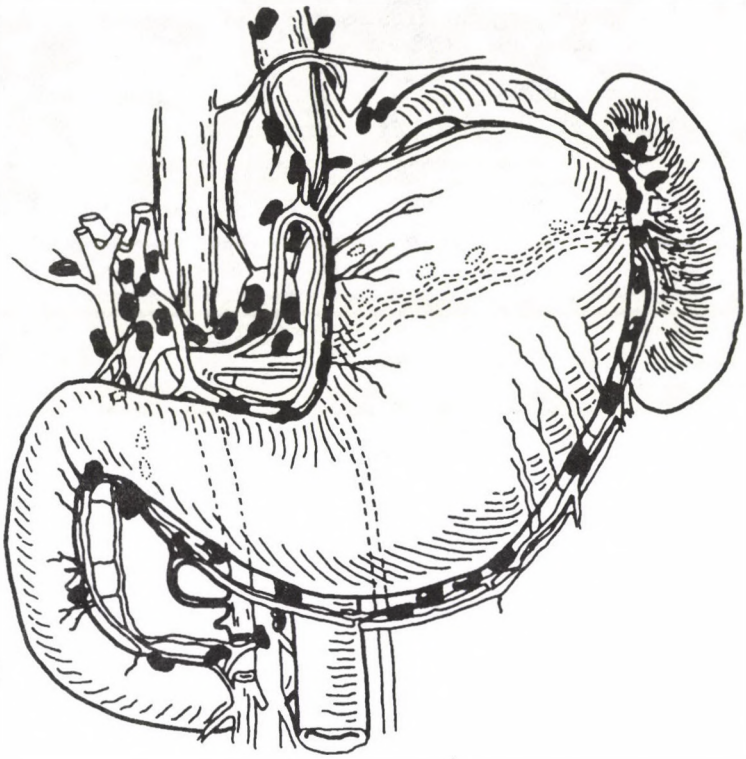

Fig. 2. Typical lymph nodes positions around the stomach

The 5-year survival rate calculated for all types of resection performed for gastric cancer is fluctuating between 12.6 and 32.4% (if palliative and radical resections are not separated from each other). The exclusive survival for radical resections ranges between 18.6 and 83.5%. The combined resection rate (without regard to the type of resection) at the Clinic of Surgery of the Albert Szent-Györgyi University Medical School (SZOTE) was published by I. Petri /37/ was 25.7% for 5-year survival and 18.3% for 10 year survival. The respective survival rates for radical surgery were 39.1 and 28.1%.

In an extensive study of the ACS two thirds of the cases belonged to stages III and IV while the 5-year survival 13.6 and 3%, respectively /49/.

It is a balanced standpoint that the location, the stage and Lauren's histological scoring present the most important data for selecting the type of surgery /13/. The histological type and the degree of differentiation are further important factors.

Subtotal gastric resection is performed when a small distal carcinoma is located in the antrum and the tumour belongs to Lauren's intestinal type. In these cases the lymph nodes of

the greater omentum, those of the lesser omentum and the lymph nodes of compartment I are removed. Splenectomy is omitted. The lymphadenectomy is extended to compartment II provided there is no remote metastasis.

We indicate total gastrectomy when the tumour is in the upper-middle third. With respect to the rapid spread of the tumour, the distal 3 to 5 cm of the segment of the esophagus is removed, just like the lymph nodes of the omentum maius and minus as well as those of compartment I. (In radical surgery even the lymph nodes of compartment II are removed.) Of course, in surgery performed with palliative purpose lymphadenectomy and splenectomy should be omitted.

If the tumour is adherent to removable organ — or part of an organ — the affected organ (part of organ) (crura of diaphragm, hepatic lobe, adrenal gland, pancreas, colon transvers colon, etc.) may be removed. This is the so-called combined surgery.

Combined surgery does not considerably increase the mortality but the chance of survival. The 5-year survival rate for combined operations was 14.2% (5-year survival) and 9.6% (10 years survival) /37/. Accordingly it is justified to perform combined surgery in every case in which it is technically possible /16, 37/.

In advanced cases, palliative resections are the most effective symptomatic treatment, too. Resection is of no reason in case of carcinosis peritonei and extensive hepatic metastases. In such cases resection may be the intervention of choice only exceptionally.

On the surgical treatment of "early" gastric carcinoma

The designation "early" is typical of the potential curability of the tumour, it is less characteristic of the tumour's size and extension. According to a recommendation of the Japanese Society of Gastroenterological Endoscopy (1962) a cancer limited to the gastric mucosa and submucosa is called early without regard to lymph node metastases.

Although early cancers are often multicentric, lymph node metastases occur as considerably high frequency. Nevertheless, the tumours are mostly curable if an adequate treatment is applied. The 5-year survival rate ranges between 60% and 100%. However, there are debatable questions referring to surgical treatment. To increase radicality, many authors indicate total gastrectomy combined with lymphadenectomy. Others are satisfied with partial resection applied with zone security.

Most of the surgeons performing distal resection recommend resection of a 5 cm healthy segment of the stomach while others perform a more extensive resection. Recurrence, though rare in occurrence, is fatal in outcome. Most of the recurrences occur within five years. They are more common (8.4%) if "early" carcinoma has already extended into the submucosa than if it is limited to the mucosa (2.2%). On the Western hemisphere, recurrence is not attributed to operations performed with insufficient radicality (resection and removal of lymph nodes). The recurrence rate is sometimes elevated if a non-early cancer is considered to be early /12/.

When early changes have been demonstrated in the proximal segment of the stomach, proximal resection is taken into account. However, such an intervention may be followed by unfavourable event. The probability of the "positive resection line" and of carcinoma remaining in the distal gastric stump is high. It is more difficult to prepare a correct lymphadenectomy, and an esophagogastrostomy after proximal resection is, so-to-say, always followed by (duodeno)-gastro-esophageal reflux and a consequent severe esophagitis. Maruyama /27/ recommends total gastrectomy with preserved pancreas.

The prognosis of the "early gastric cancer", especially of those with tumour limited to the mucosa, is very good. Kurita et al. /24/ published data of 520 cases, lymph node metastasis was found in 2.1% of 291 cases of mucosal cancer and in 15.7% of 299 cases of submucosal cancer. Neither lymph node metastasis nor permeation of the lymphatics occurred unless the lesion was large than 10 mm in diameter. In case of such circumscribed early carcinomas, limited resection, including endoscopic resection, can be performed without any risk. There is no doubt that an accurata judgement of the depth of the tumour is of basic importance when this treatment is to be chosen. Today the endoscopic ultrasonic mucosectomy provides the most reliable approach. However, even such a method fails to provide an answer of full value. Perhaps this is why Watanabe et al. /51/ recommended a combination of mucosectomy with laser treatment.

Surgical treatment of the early cancer needs the most radical intervention followed by the lowest risk: postoperative life quality should also be taken into consideration.

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LYMPHADENECTOMY IN GASTROINTESTINAL CANCER SURGERY

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(Received: September 23, 1994)

Extended lymph node dissection is an important part of the surgical treatment for cancer of the esophagus, stomach, colon and rectum. With an appropriate lymphadenectomy a more precise tumour-staging, an increase in resectability, a rise of the rate of R_0-resections, further a decrease in local tumour recurrences and an improvement of prognosis can be achieved.

Keywords: Lymphadenectomy, gastrointestinal cancer

Survey

Lymphadenectomy is a well known and proved therapeutic procedure in oncologic surgery. About 100 years ago, it was initiated as an operation for cure of breast carcinoma by Halstedt /6/, 15 years later by Miles /14/ for rectal carcinoma and by Turnbull as Non-Touch Isolation Technique for colonic carcinoma /20/. Lymphadenectomy in gastric and oesophageal cancer was standardized in Japan a few years ago and it is increasingly performed worldwide /1, 12, 13/.

The therapeutic aim of lymphadenectomy is as follows:

1. To assess the exact tumour stage.
2. To increase the resectability due to greater extraluminal radicality.
3. To enhance the rate of R_0 resections (no microscopic and macroscopic residual tumour according to the UICC 1987 classification) /7/.
4. To decrease local recurrences.
5. The ultimate purpose of lymphadenectomy is to improve the prognosis.

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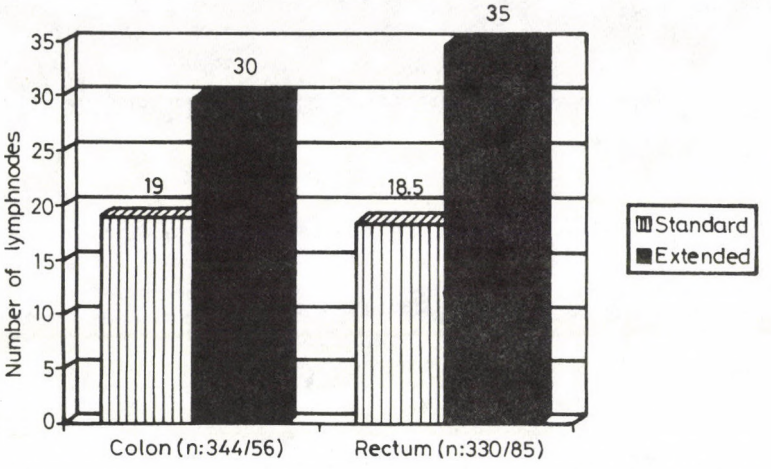

Fig. 1. Number of removed lymph nodes in patients with colonic and rectal cancer treated with standard and extended lymphadenectomy

ad 1. The assessment of tumour state is mandatory for cure in oncologic surgery. Other prognostic factors, particularly the nodal status are also related to tumour stage. For pN a reliable tumour staging is only possible with an adequate lymphadenectomy. The number of lymph nodes removed can be increased by a more extended lymphadenectomy in esophageal, gastric, colonic and rectal cancer surgery. In Fig. 1 the data of the Surgical Clinic Erlangen are shown, the number of lymph nodes can be doubled by lymphadenectomy in case of colonic and rectum cancer /5/.

The same increase can be achieved in esophageal cancer (Fig. 2). There is a new trend in esophageal cancer surgery, according to which some surgeons perform, instead of a two-field lymphadenectomy, a so-called three-field lymphadenectomy. The former includes lymphadenectomy of the lower mediastinum and the suprapancreatic area. The three-field operation extends the removal of lymph nodes above the azygos vein on both sides of the trachea along the recurrent nerves as well as in the region of the vessel sheets to dissect the cervical lymph nodes, too /10, 11, 21/.

According to recent reports /10, 11/ the rate of cervical lymph node metastasis in cases subjected to nodal dissection in three regions was almost the same, about 30%. Akiyama /1/ also reported that lymph nodes in the superior mediastinum were metastatic in 36% of the tumours of the upper thoracic oesophagus, in 12% of mid thoracic oesophageal tumours and in 11% of lower thoracic oesophageal tumours. Therefore, a three-field lymphadenectomy is advised not only for tumours of the upper oesophagus, but also for middle and lower thoracic oesophageal tumours.

The significance of an appropriate lymphadenectomy for staging purposes is indicated by the fact that the more lymph nodes are removed during surgery the more is the number of lymph nodes found positive at histology.

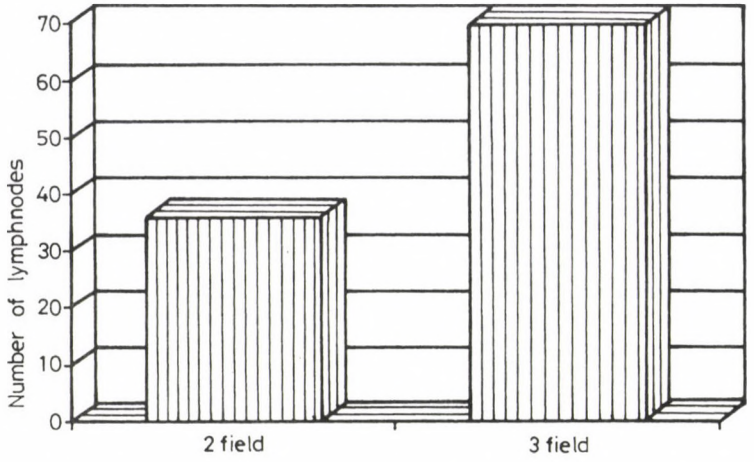

Fig. 2. Number of removed lymph nodes after two-field and three-field lymph node dissection for oesophageal cancer

In Siewert's series 53.6% of lymph nodes were positive when less than 20 mediastinal and perigastric nodes were examined, and 70.4% were positive when more than 20 nodes were examined /18/. Therefore, the stage of patients without a correct lymphadenectomy can be underestimated and hence the prognosis will be misjudged.

An appropriate example was given by Gall /4/ in connection with colonic cancer, that lymph node metastasis was present in 18% even there from where it could not have been removed by conventional resection.

ad 2. Lymphadenectomy increases the resectability rate. Siewert and Gall /4, 5, 17/ demonstrated a 10% increase of resectability in patients with gastric and colonic cancer. Resectability is a surgeon-dependent subjective category. Lymphadenectomy demands good surgical skills and with the improvement of surgical techniques there will be less and less tumours that are thought as locally inresectable.

ad 3. The most important consequence of lymphadenectomy is that it significantly increases the rate of R_0-resection. Siewert and Gall /5, 17/ reported a 8-15% growth in the rate of R_0-resections in case of stomach, colonic and rectum cancer sinthe the initiation of lymphadenectomy. Akiyama /1/ demonstrated similar results in connection with the oesophageal cancer.

In the German Gastric Cancer Study, containing the data of 2394 patients with gastric cancer, the prognostic relevance of systemic lymph node dissection was evaluated /19/. Of 1654 patients undergoing resection, 558 had a standard lymph node dissection, defined as fewer than

Table I

Tumour recurrence after standard and extended lymphadenectomy for oesophageal, gastric and rectal cancer

	Standard	Extended
Siewert: gastric cc	20%	6.9%
Enker: rectum cc Dukes C	60%	30.8%
Hojo: rectum cc		
Dukes B	21.8%	6.3%
Dukes C	32.9%	23.6%
Isono—Barbier esophageal cc	50%*	5.3%

*Transmediastinal oesophagectomy

26 nodes in the specimen. 1096 underwent radical lymphadenectomy with 26 or more nodes in the specimen. The R_0-resection was one of the strongest prognostic factors on multivariate analysis. The frequency of R_0-resections in patients undergoing standard or radical lymph node dissection related to the pT and pN categories were examined. Radical lymphadenectomy significantly increased the rate of R_0-resections in patients with pT_2, pT_3 or pT_4 tumours and in those with pN_0, pN_1 or pN_2 lymph node status.

ad 4. Lymphadenectomy as prophylaxis of local recurrences.

There is a great chance for a locoregional recurrence if lymph nodes infiltrated by carcinoma are left behind. These positive locoregional lymph nodes affect the quality of life and an additional aggressive therapy is often required. Considering this aspect, a lymphadenectomy appears to be indicated also for advanced stages, because it will help to achieve regional tumour clearance which is prognostically irrelevant yet is important for local recurrence.

Table I shows that tumour recurrence after rectum operations with lymphadenectomy was reduced to its half /3, 8/. The recurrence after R_0 oesophageal resections was 5.3% in Siewert's material /18/. This low local recurrence rate is especially relevant if compared with the rate of local recurrence following transmediastinal blunt dissection of the oesophagus. Barbier /2/ found local recurrences in more than 50% of cases following an incomplete lymphadenectomy. Siewert and Lange /17/ reported on a decrease of local recurrence from 20% to 6.9% in patients with gastric cancer treated with an adequate lymphadenectomy.

Table II

5-year survival after standard and extended lymphadenectomy in patients with rectal cancer

	Standard n:245	Extended n:192
Dukes A	87%	95%
Dukes B	61% $P < 0.05$	88%
Dukes C	43% $P < 0.05$	74%

Nakayama and Nishi /15/ analysed the type and incidence of recurrences in 2523 patients who died of stomach cancer after surgery. The most common type of relapse was peritoneal dissemination (43%), followed by haematogenous metastasis (24.9%). Surgical failure accounted for 32%, local recurrence for 18.6% remote lymphatic spread for 11%, and stump recurrence for 2.4% of the lethal outcome. These data are promising from the surgical point of view, for with adequate lymphadenectomy and radicality an improvement in prognosis can be achieved in 30% of all cases.

ad 5. The main purpose of the lymphadenectomy is to improve the prognosis. Gall /5/ compared the 5-year survival rates after extended and standard lymphadenectomy in patients with colonic and rectal cancer (Fig. 1). The 5-year survival was 80.9% versus 64.3% for colonic cancer and 66.3% versus 48.6% for rectum cancer. Hojo /8/ demonstrated a significant improvement in survival for patients with Dukes B and C rectal cancer (Table II).

Maruyama /13/ justified the usefulness of lymphadenectomy in a big stomach cancer patients collective. In the German Gastric Cancer Study /19/ radical dissection significantly improved the survival rate in patients with UICC stages II and IIIA. Radical lymphadenectomy conferred no survival advantage in patients with pN_2 tumours.

Isono /10/ has recently analysed in a Japanese collaborative nationwide study to show whether there is a prognostic benefit by the three-field lymphadenectomy as compared to the 2-field lymphadenectomy (Fig. 2). Out of 4590 resected patients 1791 were treated with three-field lymphadenectomy, 2799 by a two-field lymphadenectomy. Overall the three-field lymphadenectomy improved the 5-year survival rate up to 10% as compared to two-field lymphadenectomy. Within the single subgroups it seems that a prognostic benefit can be achieved for early stages of lymph node metastases but not for patients without or with advanced nodal metastases.

Table III

Operating time, blood loss and complications after standard and extended lymphadenectomy for rectal cancer

	Standard n:245	Extended n:192
Operating time	288 min	312 min
Blood loss	1500 ml	1900 ml
Anast. insuff.	16.9%	22.8%
Infection	8.2%	6.8%
Ileus	4.1%	4.1%
Urinary-voiding failure	8.8%	39.4%
Impotency	40%	75%

It seems that a number of patients with lymph node involvement can be cured by these techniques and, also when cure is not obtained, these procedures offer the best local control of cancer with a lower incidence of regional recurrences. However, using these techniques, postoperative morbidity and mortality are not negligible, especially in patients with oesophageal and rectal cancer. The operating time of a three-field lymphadenectomy is 7-8 h. The incidence of laryngeal nerve paralysis and tracheobronchial ischaemia is very high /16/. The postoperative morbidity and mortality rate is also higher in patients with rectal cancer treated with wide ileopelvic lymphadenectomy (Table III). Hojo /8/ reported that the radical iliopelvic lymphadenectomy was successful as for as decreasing the incidence of local recurrence and also in prolonging surival, there were increased incidences of urinary-voiding failure and sexual impotency. Extended lymphadenectomy does not increase the morbidity and mortality after resection of gastric and colonic cancer /9, 19/.

Preliminary results suggest that a more accurate surgical staging and, consequently, a more realistic long-term prognosis is provided by extended lymphadenectomy. These reasons emphasize the importance of extended lymph node dissection at least for selected carcinomas, depending upon the stage and the load-bearing capacity of the patient.

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Acta Medica Hungarica, Vol. 50/3—4, pp. 229—235 (1994)

BONE TUMOURS IN CHILDHOOD

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(Received: September 23, 1994)

The author gives a survey of literature on bone tumours in childhood and a comparison with his own work. Introducing four cases he concluded, that the survival rate of malignant bone tumours are increasing, the autologous bone transplantation seems to take priority against prosthesis in childhood, and the autologous bone transplantation is more reliable method, than the homologous one.

Keywords: Bone tumours, autologous bone graft, homologous bone graft

Introduction

Because of their more frequent appearance in children the importance of bone tumours is much higher in children then in adults. The incidence of the malignant bone tumours is in general 0.5%, and it is 5.6% under 15 years of age /1, 2, 5, 8, 9/. 5% of the solid tumours in childhood and 60% of the malignant bone tumours are osteosarcoma, which occurs most often /10/. The next largest group is Ewing sarcoma, with 3-6% in solid tumours and with 30% in malignant bone tumours /1, 11/.

The appearance of the malignant bone tumours is different in childhood from those encountered in the adulthood. Meanwhile the malignant tumours in adulthood are mainly carcinomas and their localization is lung, kidney, breast and digestion system in order, the paediatric tumours are mainly sarcomas in the haemopoietic, reticuloendothelial and nervous system, in the muscles, bones and kidneys. In childhood the localization and the development of the tumour is mainly at the place and in the time of the intensive growth.

The age distribution (in years) of osteosarcoma is as follows:

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8% in the first decade, from age 1 to 9,
60% in the first twenty years (1-19) and
32% later.

Concerning the localization, osteosarcomas are very near to the growth plate in the metaphysis of long bones, where the growth is fast (distal femoral and proximal tibial end, proximal part of the humerus and distal end of the forearm) /6/. The osteosarcoma occurs earlier in girls than in boys as it happens with bodygrowth as well. The children with osteosarcoma are much taller than the healthy ones /4/.

It is a special problem in childhood that hereditary bone dysplasias, which are tumour-like laesions (multiplex and solitary enchondroma, osteochondroma, fibrous dysplasia) can be transformed malignantly. Benign bone tumours and tumour-like laesions in childhood in the aggressive stage may require surgical intervention similar to these required by malignant diseases. Bone tumours in childhood are biologically more active and may show fast cellular growth. The soft tissue infiltration, the metastasis and the deterioration in general health are the main characteristics.

The success in the treatment of malignant tumours including bone tumours, has improved within the last 5 years. The survival rate following pure surgical intervention in the early years was around 20%, but the introduction of radio- and chemotherapy raised the slightly more than 5-year survival up to 65%. Limb saving surgery has become a reality. Children with malignant bone tumours have more chance to survive, therefore, the selection of surgical method is important, providing a good quality of life for years. The tumour surgery in childhood may be less aggressive when it is aimed at eradicating the tumour: this is equally valid for radio-, chemo- and surgical therapy. It should be keen to restore the anatomical and functional condition.

Concerning the possibilities in reconstruction the bone transplantation and the tumour prosthesis are very good examples. The reconstruction could be achieved for ever with a good bone transplantation, but the implanted tumour prosthesis in childhood requires revision in the successfully treated cases.

Materials, observations, case reports

The Hungarian Bone Tumour Register is operating in the Orthopaedic Department of our University: 80% of the primary tumour cases are treated there. The distribution of the registered tumours is shown in Table I.

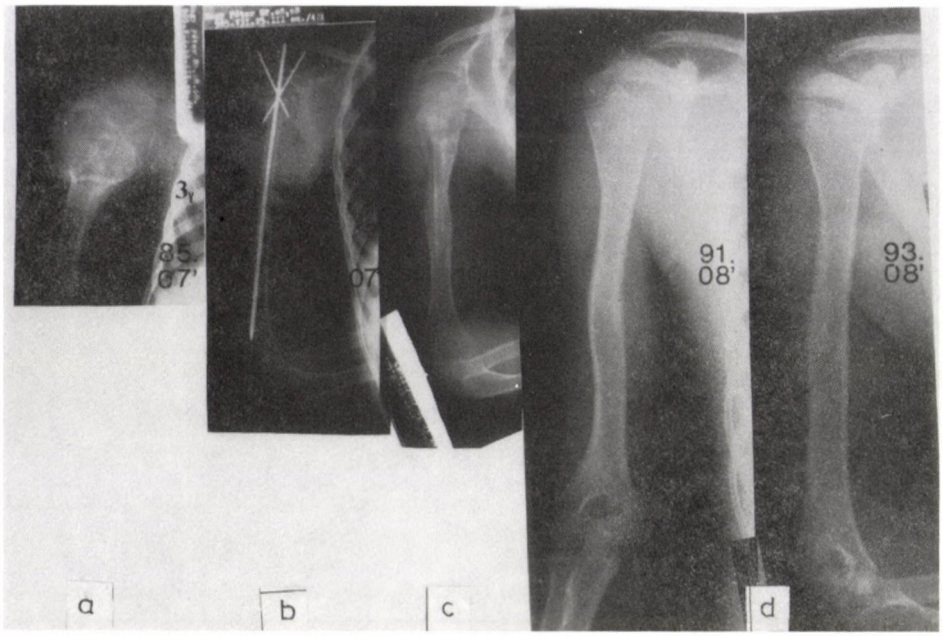

Fig. 1. a. A 3-year-old boy. Benign osteoblastoma with secondary aneurysmal bone cyst in the proximal metaphysis of the right humerus. b. Following the metaphysis was resected, the contralateral fibula segment was transplanted. c. Three months postoperatively. The fibula was incorporated. d. Six and eight years postoperatively. The appearance is the same on both sides

As it was mentioned before, in some cases of benign tumours and tumour-like diseases, radical surgical intervention may be required. The chemotherapy as recommended by COSS for patients with osteosarcoma and by CESS for Ewing sarcoma cases was performed in the IInd Paediatric Department of our University. Radiotherapy was performed in the Ewing sarcoma cases in addition. Our surgical opportunities are demonstrated in the following case reports.

Case 1. A 3-year-old boy suffered from a spontaneous fracture of the proximal metaphysis of his right humerus (Fig. 1). Aneurysmal bone cyst was suspected, therefore segment resection was performed and contralateral autologous fibula graft a successful reconstruction was achieved by. The bone incorporated well, healed with minimal abbreviation and the fibula was regrown from the periosteum. The histology showed benign osteoblastoma with secondary aneurysmal bone cyst.

Case 2. A 12-year-old boy had central chondroma in the proximal third of the left femur, it tended to transfrom malignantly (Fig. 2). The femur was resected, and we were able to preserve the proximal metaphysis, assuring the autologous fibular transplantation stabile. Following the postoperative procedure, orthesis was applied. Playing football after the orthesis was unlocked and the fibular graft was fractured (Fig. 3). The fracture was healed in plaster. The child grew tall and the affected limb was 5 cm shorter than the opposite one, consequently, the right leg was shortened. The final result was a 175 cm tall child, with the same leg length.

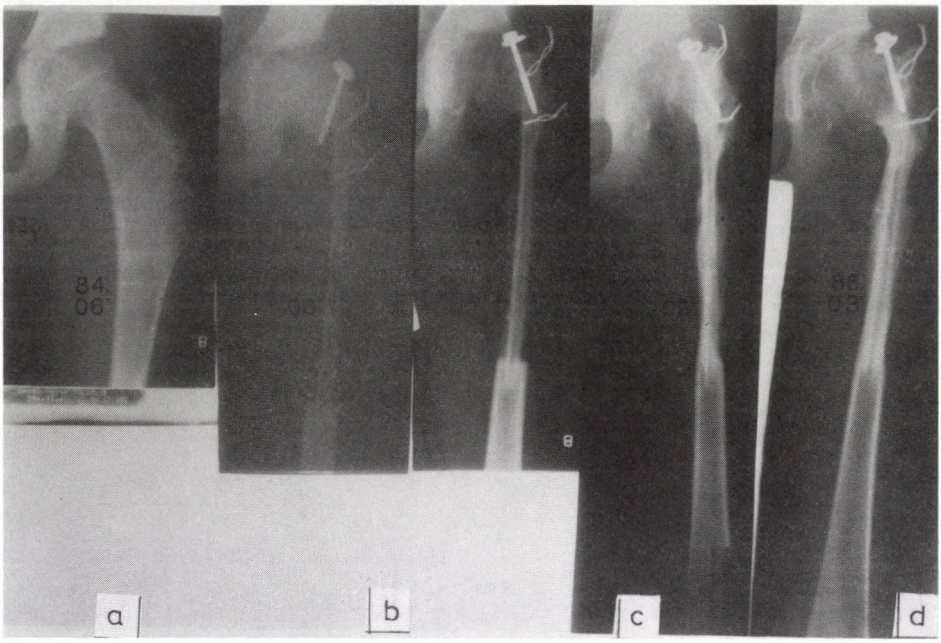

Fig. 2. a. A 12-years-old boy. Chondroma in the proximal metaphysis of the left femur. b. Resection, fibula transplantation. c. Three months postoperatively.Signs of incorporation. d. One year postoperatively.Remodellation, thickened fibula

Case 3. A 13-year-old boy with intracortical osteosarcoma in the distal and lateral part of the left tibia (Fig. 4). Following enlarged resection the bone was healed and the remodellation was complete. After 8 years surgery the patient was symptomfree. This was the case, in which the moderate aggressivity lead to success.

Case 4. A 14-year-old girl with osteosarcoma in the distal third of the left tibia (Fig. 5). Unfortunately the case due to enlarged tumour was not suitable for limb saving surgery, but because of the parents' objection against amputation, radical resection and fibula transplantation were carried out. The fibula incorporaiton was good, but, due to recurrency in the contralateral knee and lung metastasis, the patient was lost 2 years after the operation.

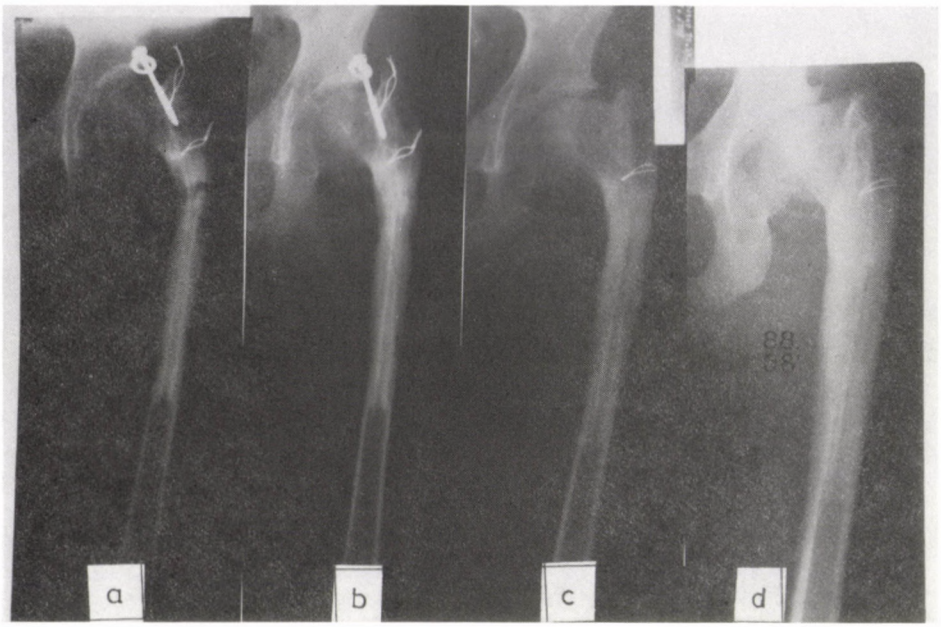

Fig. 3. The same patient. a. Fractured transplanted fibula. b. Healing in plaster. c—d. Two and four years postop. The transplanted fibula is enlarged, complete recanalization

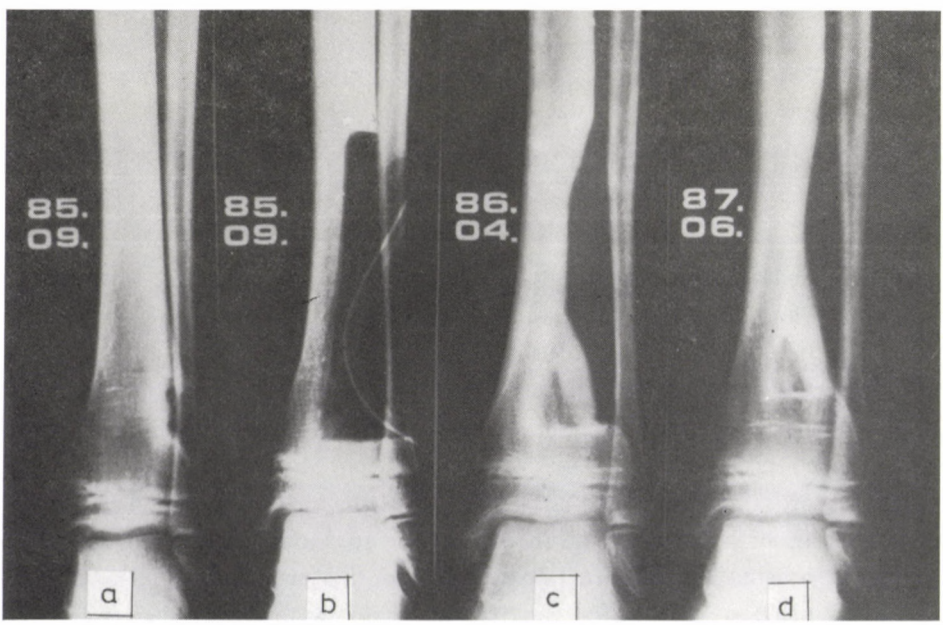

Fig. 4. a. A 13-year-old boy. Intracortical osteosarcoma in the distal metaphysis of the left tibia. b. Large resection. c—d. One and two years postop. Fast restitution

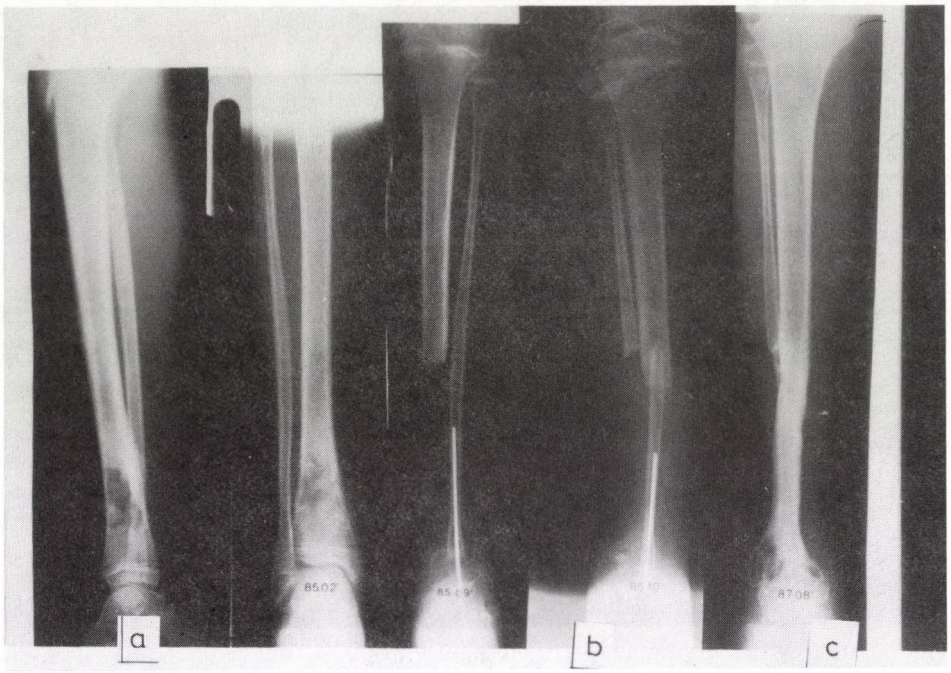

Fig. 5. a. A 14-year-old girl. Osteosarcoma in the distal metaphysis of the left tibia. b. Radical resection, fibula transplantation. c. Good incorporation, full weight-bearing

Discussion

According to the literature and concerning our results the survival rate of the malignant bone tumours has been increased due to the modern multidisciplinary co-operation in the treatment.

In paediatric cases, survival time has been prolonged, so the present problem to be solved has been shifted to the old age. The bone transplantation, which requires more co-operation and more time, seems to take priority against prosthesis.

In the meantime, papers have been published about success with homologous bone and cartilage-bone transplantation, and the autologous bone transplantation seems to be a more reliable method. Fortunately, we have two fibulae, so, using either one or both, augmented with cancellous bone from the iliac creast, we are able to solve most of the problems.

The problem could be solved for ever using autologous bone transplantation, and the chance doing any other correction (e.g. elongation) is still there.

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Acta Medica Hungarica, Vol. 50/3—4, pp. 237—244 (1994)

NEW ASPECTS IN THE TREATMENT OF BONE SARCOMAS

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(Received: September 23, 1994)

The author reports on the progress made in the treatment of bone tumours in the last two decades. There is a short description of new entities like the solid form of aneurysmal bone cyst; dedifferentiated and clear-cell chrondrosarcoma; low malignant central osteosarcoma; periosteal and high-grade surface osteosarcoma, which have recently been reported in the literature. The response to the chemotherapy in osteosarcoma and the problems of limb-saving surgery in bone tumours are discussed.

Keywords: Bone tumours, chemotherapy, limb saving surgery

Survey

Bone tumour centres, international collaborations

The last two decades have brought significant changes in the treatment of bone sarcomas. In the past, the histology and the surgical treatment of bone tumour patients were performed in the pathological and surgical departments of general hospitals. Considering the moderately frequent occurrence of these tumours — e.g. the incidence of osteosarcoma is about 1.5/million/year — our efforts failed in the diagnostic procedure and treatment. This problem was first recognized by Codman, who founded subsequently the Bone Sarcoma Registry in 1920 in the USA. The aim of this Registry was the histological classification of tumours besides recording data. Great personalities, like Phemister, who distinguished the chondrosarcomas from osteosarcomas; Parker and Jackson, who described the reticulum-cell sarcoma of the bone in 1939; or Ewing, who characterized his round-cell sarcoma on basis of 2000 retrospectively analysed bone tumours, hall-marked this work.

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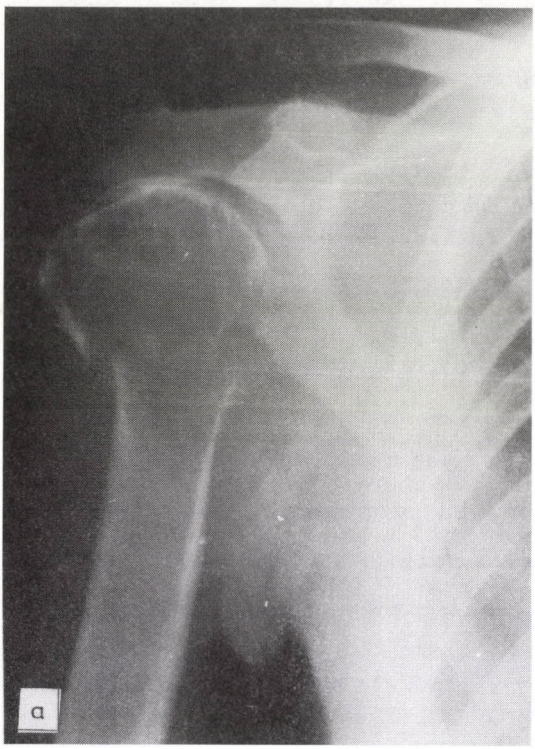

Fig. 1a. Solid form of aneurysmal bone cyst. Pure lytic lesion with pathologic fracture of the humerus (anteroposterior view)

Bone tumour registries were founded later in many countries; specialized radiologists, pathologists and oncologic surgeons have been working in close collaboration in Bologna, Vienna, Paris, Birmingham and Prague, only to mention the greatest centres in Europe.

Considering the low number of the newly-recognized bone tumour cases (5-9 Ewings' sarcomas, 1-3 malignant fibrous histiocytomas of the bone a year in a 10 million population), there has been an increasing demand to establish international working-groups. This is how the Arbeitsgemeinschaft für Knochentumoren was founded in the German-speaking world twenty years ago and the European Musculoskeletal Oncology Society (E.M.S.O.S.) in 1987. The Bone Tumour Registry of our University is founding member in both of the working-groups. The aim of the international collaboration is to characterize the clinicopathological features of rare bone tumours, to elaborate new diagnostic and therapeutic schedules and to evaluate the treatment results on the basis of unified criteria.

Within the international collaboration the Arbeitsgemeinschaft für Knochentumoren reported on 25 adamantinoma cases and the E.M.S.O.S. evaluated the treatment results of 677 giant-cell bone tumours /2/.

Fig. 1b. The cut surface of the resected specimen presents a solid tumour

Progress in the classification of bone tumours

The implementation of new immunohistochemical techniques and the careful evaluation of large series of bone tumours resulted in description of new tumour entities.

Recently, the solid form of aneurysmal bone cyst has been described /10/. The X-ray appearance of the pure lytic-cystis lesion is not characteristic (Fig. 1a, b), it is easily mistaken for a giant-cell tumour or at least for an osteosarcoma. The solid variant of the aneurysmal bone cyst is composed of a fibroblastic pseudosarcomatous and fibrohistiocytic stroma in which abundant osteoclasts and newly-formed bone trabecules are present.

Very rare types of the chondrogenic bone tumours are the defifferentiated and the clear-cell chondrosarcoma, both of which were first reviewed in the early seventies /1, 3/. The radiographic and histologic appearance of the latter form is very similar to that of the chondroblastoma. Among the characteristic tumour cells with clear cytoplasma and small round dark

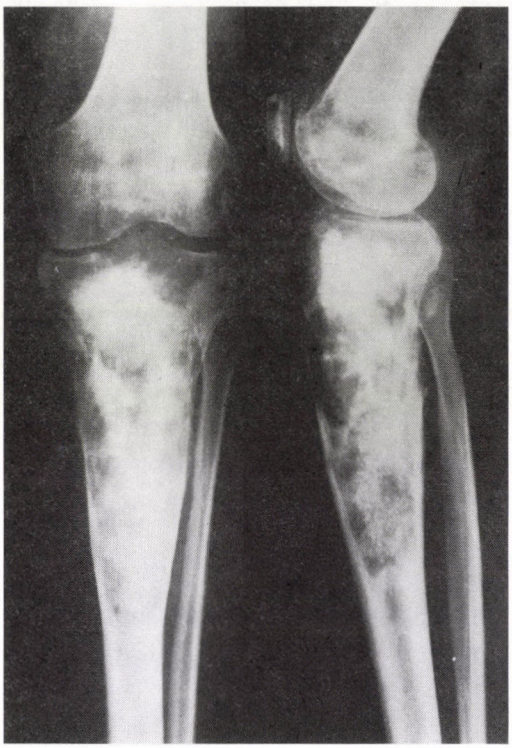

Fig. 2. Low malignant central osteosarcoma; fibrous dysplasia-like lytic-sclerotic destruction of the proximal tibia

nuclei situated centrally there are numerous multinucleated giant cells scattered through the stroma. Metastases occur seldom, survival is favourable.

A new entity of the osteogenetic bone tumours is low malignant central osteosarcoma /14/. This rare form of osteosarcoma is well-differentiated, so that it is often mistaken for a benign process such as fibrous dysplasia or non-ossifying fibroma (Fig. 2). The local recurrence rate is high, metastases, however, appear rarely. Only the more cellular fibrous stroma and mitoses refer to the malignancy of the tumour.

The so-called periosteal osteosarcoma is often not easy to distinguish from parosteal osteosarcoma /13/. Both of them grow on the surface and destroy the cortical bone in an advanced stage. In contrast to the parosteal osteosarcoma, which affects the metaphysis of long bones and grows slowly, producing a large amount of bone, the periosteal osteosarcoma occurs on the surface of the diaphysis and it is composed of lobules of cartilage with

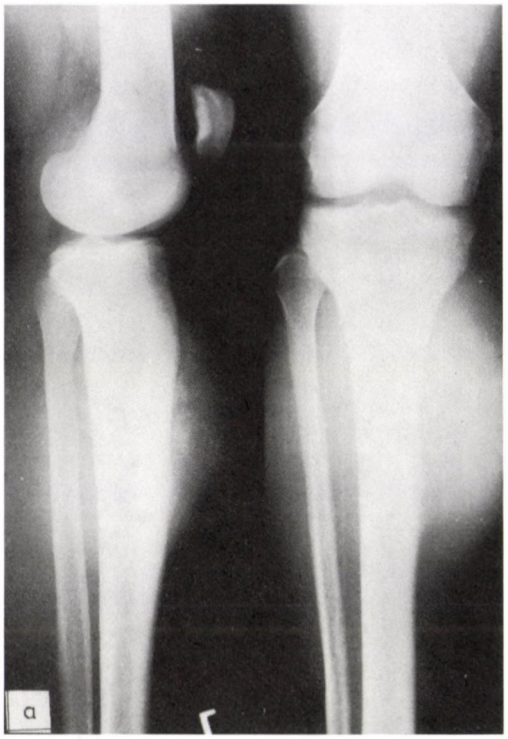

Fig. 3a. Periosteal osteosarcoma: minimal destruction of the cortical bone and wide soft-tissue extension (anteroposterior and lateral view)

central bone formation. The prognosis is better than in conventional osteosarcoma but worse than in parosteal osteosarcoma (Fig. 3a, b).

The high-grade surface osteosarcoma is the least common of the osteosarcomas. It occurs in young patients and presents the same histological features as the conventional osteosarcoma. The prognosis is poor, the soft tissue invasion is usually extensive even if the cortical bone is less involved.

Response to chemotherapy in osteosarcoma

Some decades ago osteosarcoma was thought to be a tumour resistant to chemotherapy. Rosen et al. /7/ first reported on the efficacy of chemotherapy in osteosarcoma based on high-dose methothrexate and adriamycin in 1974. The ratio of lung metastases decreased and the five-year survival rate changed from 20% to 70%, even when limb-saving surgery was performed. Also

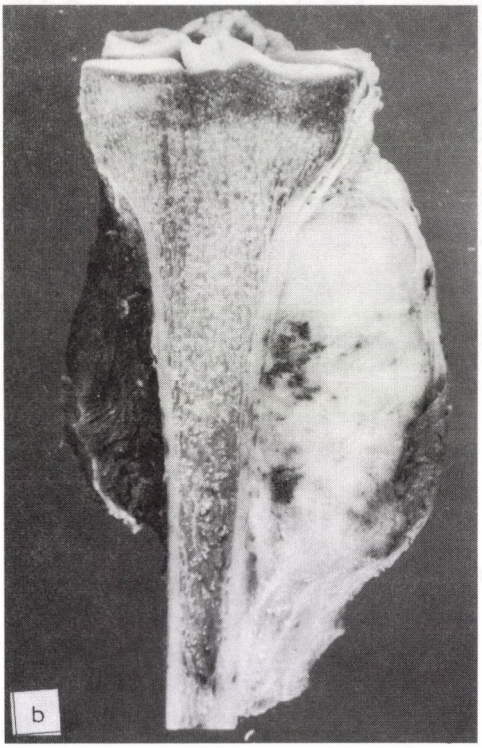

Fig. 3b. The surface of the half-cut specimen: the intramedullar cavity is not invaded by the tumour

Rosen et al. /8/ pointed to the beneficial effect of the preoperative (neo-adjuvant) chemotherapy. Shrinkage and demarcation of the tumour occurred; metastasis formation due to the procedure of the biopsy was prevented; the chemotherapy-sensitivity of the osteosarcoma can be estimated by histological evaluation following the definitive surgery.

The chemotherapy will be effective if more than 90% of the tumour cells appeare to be destroyed histologically. Our Department has been using the morphological evaluation of the chemotherapy-sensitivity in osteosarcoma since 1984. According to literary data /4, 12/ we found a close relation between the histological subtypes, the response to chemotherapy of the osteosarcomas and the five-year survival rate. Fifty to 60% of the osteosarcomas are well-responder and have a significantly better prognosis /9/ than the non-responder ones.

Changes in the surgical strategy of bone tumours

Some decades ago ablative surgery played the main role in the treatment of bone sarcomas. In spite of this, not more than 20% of the patients survived the first 5 years. Due to the effective chemotherapy, 50 to 75% of the osteosarcoma and Ewing's sarcoma patients have a chance for survival /6, 11/, even in limb-saving surgery. In 50 to 70% of the cases, limb-saving surgery has became a real alternative for amputation. The basic condition for limb-spearing was the development of reconstructive surgical techniques. Basically, the large defects of the bone following tumour resection can be reconstructed either by auto- or homografts or by tumour endoprostheses. The incorporation and union of (vascularized) autografts are superior to all other methods, they, however, can only be used as intercalary grafts for replacing dia-metaphyseal defects in most cases.

Joint surfaces can be replaced by osteoarticular homografts. Many different preserving methods (irradiation, autoclave procedures, deep-freezing in glycerol, etc.) are used in bone banks — that means that no one single procedure allows either complete incorporation of the graft into the host bone or complete survival of the cartilage cells at the surface. These massive osteoarticular grafts always remain "dead" bones and, as a consequence, complications like malunion, facture or infection occur in a high percentage of the cases.

Tumour endoprostheses can also serve the reconstruction of joint and bony defects /5/. They are custom-made or modular type. The latter one has the advantage that the exact size of the prosthesis can be estimated just after the resection of the tumour at the operation table. Modular types of endoprostheses were developed in the late seventies /5/; we have been using our own design for 5 years. Tumour endoprostheses have the advantage that they allow a full-range motion of the joint and the extremity can be weightened just after the implantation. Unfortunately, however, the risk of the prosthesis loosening increases rapidly after the first years, and an early reoperation is necessary.

It is still an open question whether bone grafts or tumour endoprostheses should be used for the reconstruction. As mentioned above both of them have many advantages and disadvantages, too. The surgical strategy depends on many factors; e.g. on histological grading of the tumour, on the surgical stage of the tumour, on the localization, on the age and general condition of the patient, on the possibilities and experience of the sur-

geon. According to our experience, we replace the defect by bonegraft in young patients with favourable life expectancies and we use tumour endoprostheses in older patients with less favourable survival chances.

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Acta Medica Hungarica, Vol. 50/3—4, pp. 245—256 (1994)

OSTEOPOROSIS — A MODIFYING FACTOR OF SURGICAL TREATMENT

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(Received: September 23, 1994)

Increasing knowledge of fragile bone has been gained by non-invasive mineral assessments. Its future importance seems to be twofold. First by, it seems likely that patients with low bone mineral will be treated to try to increase this bone mineral or at least to keep it steady. Secondly, in the presence of osteoporosis special stragies must be taken into consideration. In many locations osteoporotic fractures may need special solutions. It seems that osteosynthesis with plates and screws which have their definite indications in younger patients may be replaced by alternatives, such as cerclage wireing and intramedullary implants. Polymethylmetacrylate has been a good adjunct to strengthen screw fixation and to fill defects after compression of fragile cancellous bone.

Keywords: Osteoporosis, fracture, operative procedure

Introduction

This review focuses on the role of bone fragility in the treatment of fractures and other orthopaedic phenomena. There are different aetiologies of bone fragility, notably factors, such as age, sex, race, life habits, falling tendency and diet. Non-invasive assessment of bone mineral is assigned increasing importance in the understanding of osteopenic fractures. Bone is called fragile when it breaks under less than a normal load. In this review, bone fragility and osteoporosis are used synonymously.

Two types of involutional osteoporosis have been delineated. Postmenopausal osteoporosis is designated Type I, whereas senile osteoporosis occurring in women and men alike, Type II. A combination of the two types affects drastically the fracture epidemiology drastically.

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Several studies have shown that the strength of bone is related to its mineral content. Reduced bone mineral in osteoporosis reduces the holding power of implants. Bones are elastic and they adapt to the loads of daily life by recoiling. If the load exceeds the yielding point, permanent deformation and fracture ensure. However reduced bone mineral is the main cause of susceptibility to fracture in the elderly. In age-related osteoporosis both cortical and cancellous bone mineral are reduced /2, 4, 6/.

Problems of the treatment of osteoporotic patients

The treatment problem in an osteoporosis fracture is twofold. Bone fragility reduces the possibilities of secure fixation and the old patient may be debilitated, thus the risks of operation will be increased. Therefore osteoporotic fractures in the elderly are often treated by sed methods. The literature is poor in systematic clinical studies of the effects of osteoporosis on internal fixation, and special strategies for treating fragila structures are seldom applied in preoperative planning. Extreme bone fragility is often experienced as an unexpected, disastrous situation intraoperatively; screws do not hold, bone is splintered during reduction and fixation attempts. Special solutions have been suggested for such situations. Screws can be fixed with nuts, cerclage wireing may hold where screws loose their grips. In extreme situations polymethylmetacrylate cement has been suggested /5. 8/. The problem is the same with impaired implant fixation in osteoporotic bone. Reduced bone mineral in osteoporosis reduces the holding power of implants /8/. There is a relationship between the severity of osteoporosis and the fixation strength of pedicle screws. The pullout force is doubled by bone cement.

Surgical procedures modified by osteoporosis

The operations on the upper extremities never cause difficulties, like those on the other parts of the body. There is no weightbearing at the fracture of the distal end of the radius. However differentiated treatment of the radius is obviously required.

The most common types of osteoporosis fracture are the fractures of the hip. Stable fractures may be treated more simple than communited ones.

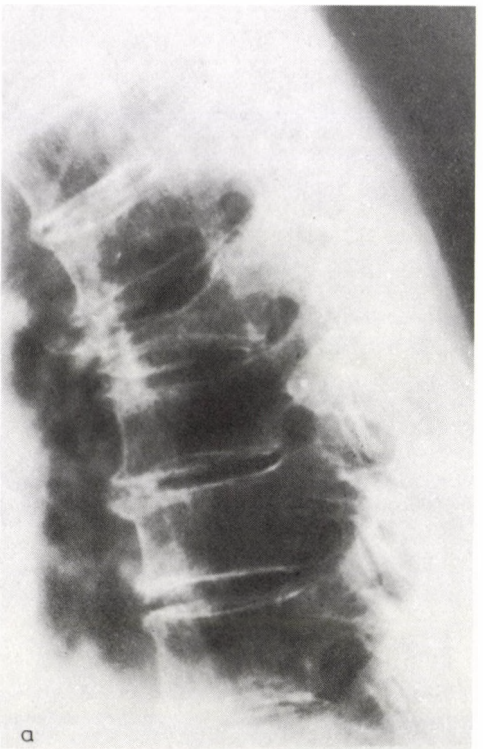

Fig. 1a. Gravis osteoporosis of the dorsal vertebras

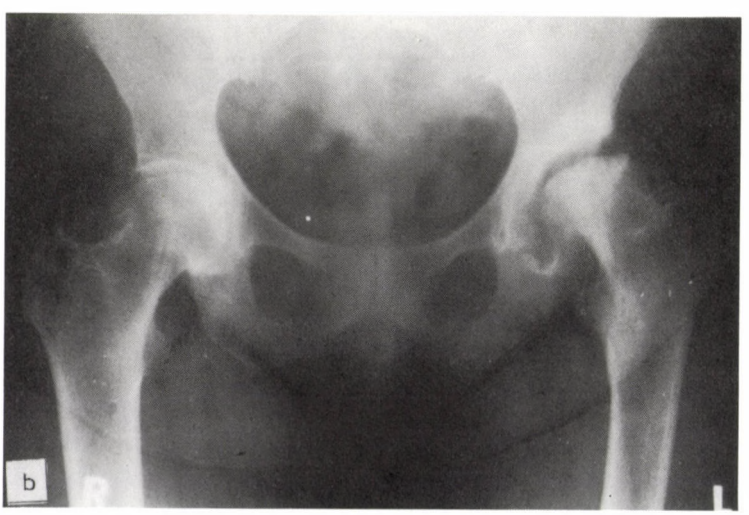

Fig. 1b. Avascular necrosis of the head of the left hip

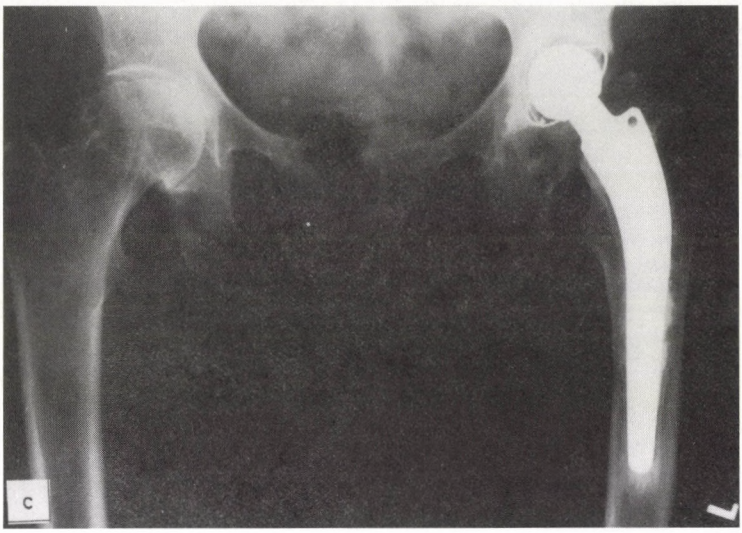

Fig. 1c. Total cemented hip replacement

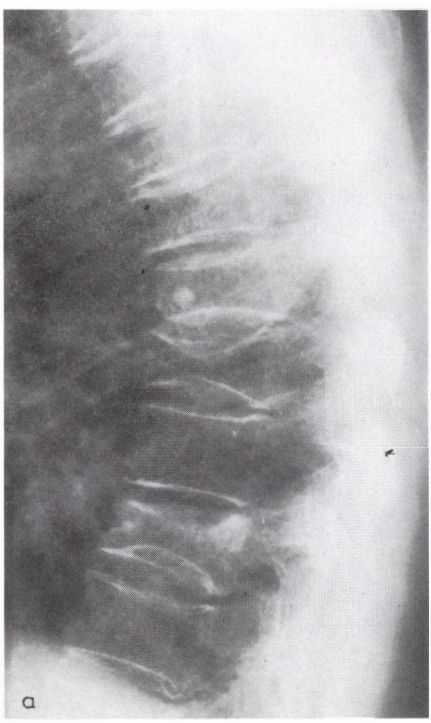

Fig. 2a. Osteoporosis of vertebrae

Fig. 2b. Osteoarthritis of the hip

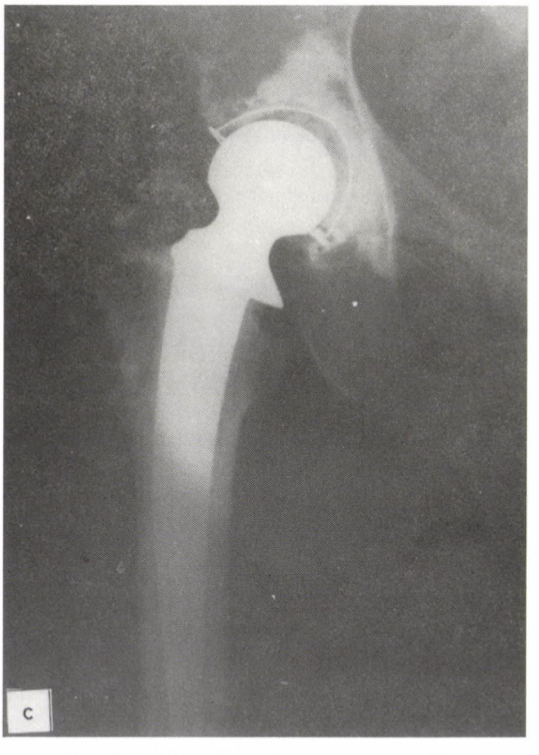

Fig. 2c. Hip endoprosthesis was inserted

Fig. 3a. Tumour-like condition of the right lateral tibial condyle. Histological examination revealed osteoporosis

In communited hip fractures intramedullary polymethylmetacrylate has been used for fixation of the endoprosthesis and the results seem favourable, therefore endoprosthetic replacement has been recommended, even in young patients. Besides early weightbearing it allows good periosteal healing (Fig. 1a, b, c, Fig. 2a, b, c).

The intramedullary location of cement also allows good periosteal healing. It seems that fractures of the distal end of the femoral shaft in the elderly may do better after intramedullary nailing than after an angled bladeplate osteosynthesis.

In knee surgery the presence of osteoporosis must be also taken into consideration. Reduced bone mineral content in osteoporosis may cause defects — e.g. compression of fragile cancellous bone. In one of our tumour-like cases bone defect was detected in the right lateral tibial condyle. Histological examination revealed the presence of osteoporosis. For such a good alignment of the femoro-tibial axis varus femoral osteotomy was carried out (Fig. 3a, b). On the other hand, at the procedure of replacement it seems to us reasonable to use bicondylar rather than unicondylar prosthesis. The unicondylar type can sink more easily into the porotic underlying bone.

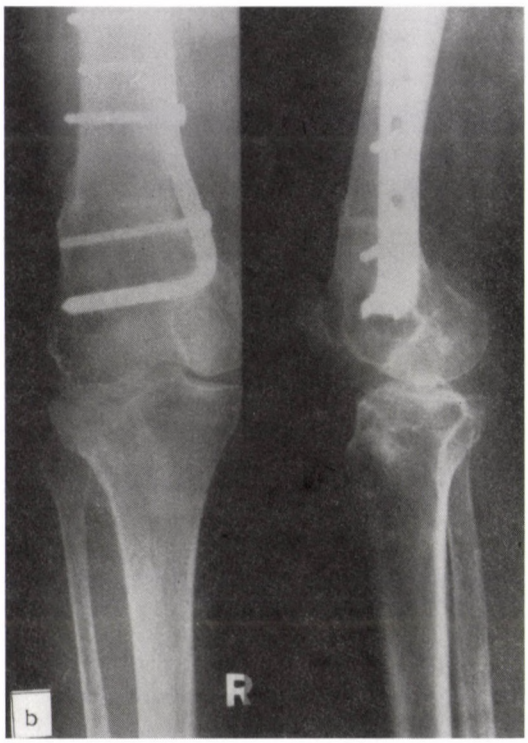

Fig. 3b. Varus femoral osteotomy was carried out

In the other case bicondylar knee prosthesis was loosened within ten months following operation (Fig. 4a, b).

In spine surgery of osteoporosis special strategies must be taken into consideration. The special nature of osteoporotic bone that limits the choice of fixation methods should be clearly understood. The fixation of short segments by pedicular screws and plate seems to be riskful in osteoporatic patients. Stability in porotic vertebral coloumn can be only achieved by fixation of longer segments (CD or USIS instrumentation, etc.) (Fig. 5a, b, c, d).

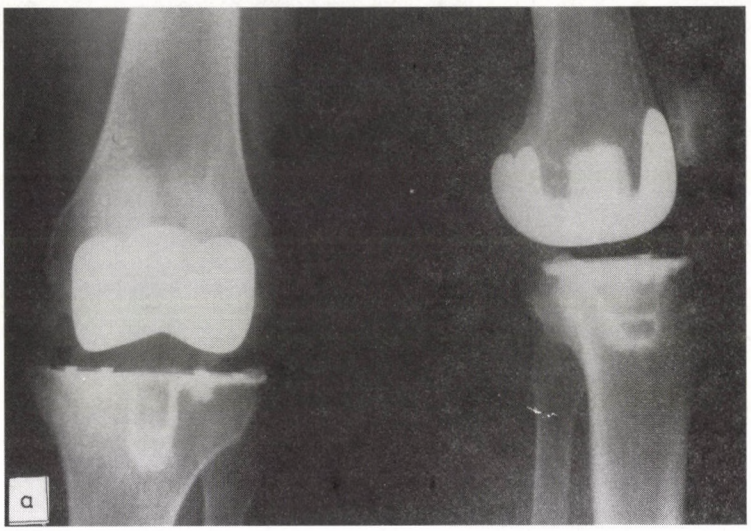

Fig. 4a. Bicondylar knee prosthesis in good position

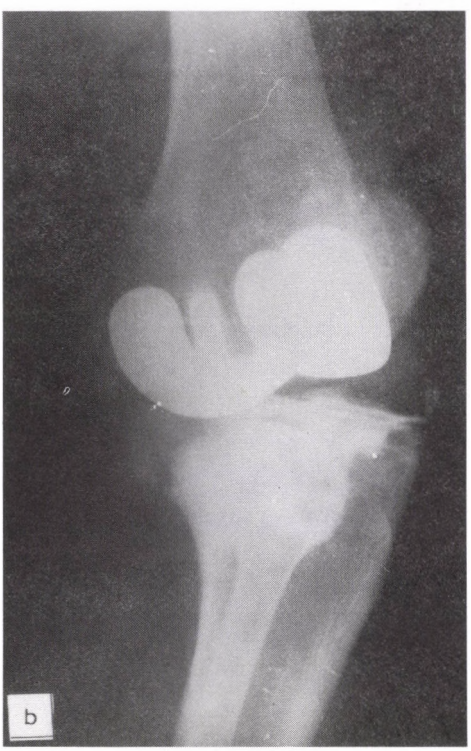

Fig. 4b. Loosening of the prosthesis

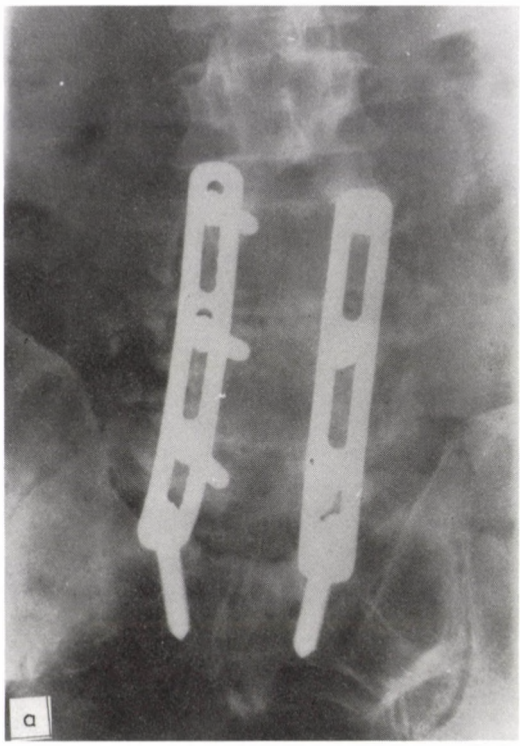

Fig. 5a. Steffe instrumentation for stabilization of the lumbar spine (anteroposterior view)

Conclusions

The patients with low mineral content should be treated to try to increase their bone mineral. Orthopaedic procedures may need special solutions according to the extreme bone fragility. Internal fixation should be combined with adjuvants as bone cement or cerclage.

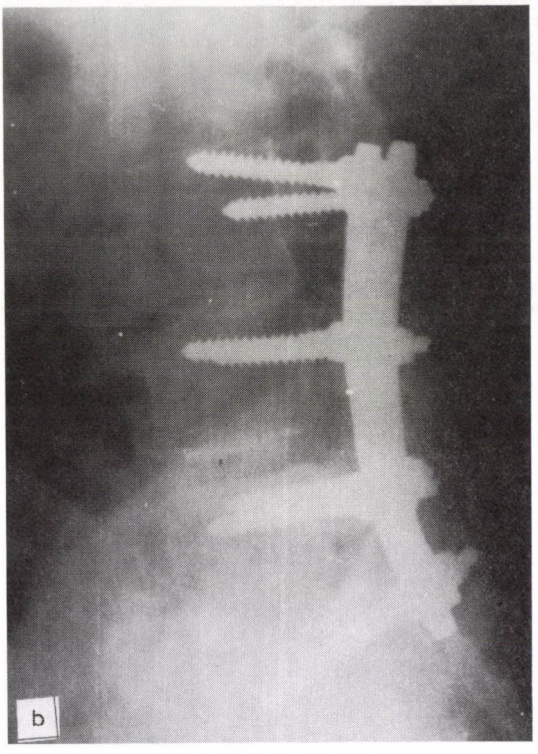

Fig. 5b. Lateral view

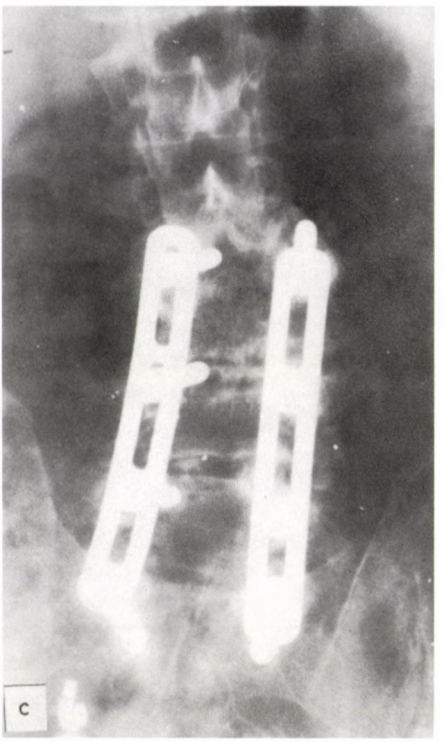

Fig. 5c. The fixation of pedicular srews is poor (anteroposterior view)

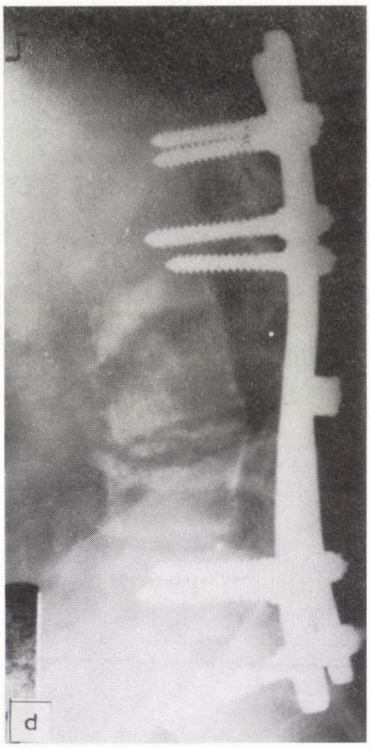

Fig. 5d. Lateral view

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Acta Medica Hungarica, Vol. 50/3—4, pp. 257—273 (1994)

CYTOKINES IN THE TREATMENT OF MALIGNANCIES

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(Received: September 23, 1994)

Cytokines are pleiotropic peptides produced by lymphoid cells that play important roles in cellular proliferation and multiplication. Diminished or enhanced production or constitutive secretion of cytokines contributes to the aetiology and pathogenesis of several diseases. They are soluble mediators eliciting specific responses of different target cells of paracrine, autocrine and cascade systems of the organism. Their secretion is regulated at the molecular genetic level. Gene rearrangements of cytokines and their receptors have been demonstrated in several diseases. As means of specific or supportive therapy, cytokine treatment has been used both in neoplastic and other proliferative diseases. Lymphokines and interferons comprise the first, whereas colony stimulating factors and growth factors yield the second group of cytokines. Most scientific experience is with interferon-alpha. Its anti-viral mechanism of action has been extensively studied and clarified, whereas its antitumour effect is more obscure and is a result of many simultaneous biologic events.

Keywords: Cytokine, interferone, interleukine, TNF, antibody against IFN-alpha

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Abbreviations: ALL: Acute lymphoblastic leukaemia, AML: Acute myeloblastic leukaemia, CAH: Chronic agressive hepatitis, CML: Chronic myelocitic leukaemia, DTH: Delayed tissue hypersensitivity, EGF: Epithelial growth factor, EPO: Erythropoietin, FGF: Fibroblast growth factor, G-CSF: Granulocyte-colony stimulating factor, GM-CSF: Granulocyte-monocyte-colony stimulating factor, HBV: Hepatitis B virus, HCL: Hairy cell leukaemia, HCV: Hepatitis C virus, HDV: Hepatitis D virus, HES: Hypereosinophilic syndrome, ICAM: Intercellular adhesion molecule, IFN: Interferon, IG: Immunglobuline, IL: Interleukine, IlGF: Insulin like growth factor, LAK: Lymphokine activated killer, M-CSF: Monocyte-colony stimulating factor, MM: Myeloma multiple, PCL: Plasma cell leukaemia, PDGF: Platelet derived growth factor, PRV: Polycythaemia rubra vera, SIRS: Systemic inflammatory response syndrome, TAC: T-cell activation, TGF: Transforming growth factor, TNF: Tumour necrosis factor, VGF: Vascular growth factor

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Introduction

Cytokines are protein-like small molecules produced by lymphoid and non-lymphoid cells. By mechanism of action they are soluble mediators eliciting specific responses in various cells in paracrine and autocrine systems or both and in pathways of the cascade reactions. The first group of cytokines is comprised of the lymphokines and interferons (IFNs), whereas the second group includes the colony-stimulating factors. Cytokines are used in diseases characterized by malignant cell or rather malignant tissue proliferation partly as casual therapeutic agents and partly as supportive therapy applied in order to alleviate undesirable side-effects resulting from other therapies. Although reports on their efficacy as casual therapeutic agents are highly controversial and there are controversies as for the latter application, we may give account of considerably good results in the latter field too.

Subjects

Cytokines are actually special forms of pleiotropic proteohormones exerting their action within a "communication network", thus, playing an important role in the complex system of cell growth and cell-functioning. Under- or over-production of cytokines results in various pathological conditions. Their regulation is mediated via genetic factors hence in various pathological states the genetic transposition has been proved, namely, genes of alpha and beta IFN get wiped off /12, 34/.

Inadequate transcriptional activity (IRF 1) of the IFN-inducing gene seen in leukaemia and myelodysplasia (preleukaemia!) could be traced to an abnormality in chromosome 5q.

Until today about a hundred such molecules — corresponding in structure and function to the above — have been described. Table I gives a classification from the point of view of clinical practice.

Cytokines as ligands and inducers can exert their influence only in the presence of their receptors to which they get attached. The mode of this attachment is not indifferent and as a consequence it influences their mechanism of action /41/. According to our present knowledge, it works as a signaling system affecting ultimately cell-level processes.

Table I

Classification of interleukines

Interleukines	IL 1-12
Cytotoxic factors	TNF-α,β
Interferons	IFN-α,β,γ,ω /22, 23/
Colony-stimulating factors	G-CSF, GM-CSF, M-CSF, EPO, IL-3
Growth factors	**EGF** (EGF, TGF-α, VGF), **FGF-α**, **FGF-β**, **TGF-β** (TGF-β-1, TGF-β-2, TGF-β-3, Inhibines, MDIP, BMP), **PDGF-1**, **PDGF-2**, **FGF-α**, IL-2, **FGF-β**, **IIGF-1**, **IIGF-2**, **IL-1** (IL-1-α, IL-1-β), **HST-2**, **Bombesin**

Apart from a few exceptions (IL-1, TNF, IFN-alpha, IFN-beta), all cytokines have their own specific receptors to which they will attach themselves and they will not compete with other cytokines for binding sites. Their binding affinity is usually high (Kd = 10^{-11}—10^{-10} mol/litre). Due to this high affinity, 10-100 ligands attached to receptors are sufficient to produce an activity /33/.

There are two important characteristics of these receptors, i.e. their expression could be induced and they have the ability to modulate upon the effect of their own or other ligands. Resting lymphocytes exhibit either few (e.g. IL-4R) or undetectable number (e.g. p55, IL-3R) of various cytokine receptors, however, these could increase in number upon the effect of cytokines /47/.

The receptor found on the surface or on the membrane of the subcellular organelle is not identical in the case of many ligands. For example, IL-2 may be bound by three molecules, however, the affinity of each molecule is

different. As a consequence, we may say that the fact of binding in itself would not always result in an identical effect. At the same time, genomes of the molecules acting as receptors (p. 55, p. 70, and the two together) seem to be entirely different from each other /31, 36/.

IL-4 and IL-5 play an important role in the expression of receptor molecules. These were examples to show that even the receptor itself is not necessarily a homogenous molecule and the extent of its expression is a function of the presence of other cytokines. However, there is an observation that in case IL-4 binds to its own receptor, IL-2R expression is not enhanced further, in fact it is rather decreased.

The foregoing allow us a glimpse into this complicated system working in a paracrine fashion whereas the techniques accumulated in molecular biology allow it to be influenced. The use of purified recombinant cytokines in infections and malignant diseases or pathological conditions with bone marrow failure and immune deficiency is not unanimously, but widely accepted /6, 35/.

Specific actions of anti-cytokine antibodies prompt us to look for therapeutic perspectives. It has been proven that generalized endothelial activation is the result of cytokine action. This state has been called SIRS (systemic inflammatory response syndrome). It should be noted that cytokines have no role in the maintenance of normal homeostasis /13/. At the same time the above described syndrome can be prevented by TNF, IL-1 /13/ and antibodies to IL-8 /2/.

Blocking of TNF by monoclonal antibodies /1, 16/ for the prevention of E. coli-induced endotoxic shock has been tried. However, for practical use soluble TNF receptors would have a more promising future — though it should be emphasized here that the intervention is of use only prior to TNF binding as afterwards the chain reaction cannot be stopped any more /3, 46/. Unfortunately, its use for prevention has not been approved even though it is known that cytokines play no role in normal homeostasis. In case of generally decreased immune response, preventive administration of soluble TNF receptor preparation may lead to death /8/.

Concluding from the above we may say that there are mechanisms to enhance both the paracrine and, in some respect, the autocrine reactions (cytokine synergism, cascades, enhanced expression of homologous and non-homologous receptors, antigen-dependent cytokine synthesis for restriction or control of all, decreased receptor expression, cytokine antagonism or switch-control). It is a great challenge for the clinical practice which

rationale to follow: enhance or decrease immunoreactivity by influencing the endogenous cytokine synthesis or cytokine administration? Anyhow, gaining more experience is essential. Most empirical data round the world have been collected regarding IFN-alpha. We, too, feel entitled to express our opinion in this context.

It was 37 years ago that a significant biological activity of IFN-alpha was first described in case of malignant processes, nevertheless, the mode of action of this cytokine still remains unknown /11/. Best results were seen in connection with haematological disorders /48/.

The antiviral property of IFN-alpha has been more or less clarified /29/ by now. IFN-alpha promotes the endonucleases and the production of 2-5 oligoadenylate synthetase, and these proteins in turn lead to the destruction of RNA-neg virion. On the other hand, it hinders protein colonization, e.g. the production of cytochrome p. 450 enzyme 42.

Many papers have been published in order to clarify the mechanism of action of IFN-alpha, however, putting together the puzzle bits still does not allow us to obtain a coherent view of the subject. Decreasing or inhibiting and increasing or augmenting effects are summarized under "latest results" in Tables II and III.

The two tables which are far from complete present clinically significant conclusions, far from being complete. Like other therapies, IFN administration has side effects. However, to label it as having "undesirable side effects" would not be correct, as, in case of a biologically active natural substance, its side effects are in fact a proof of its activity /24/.

The expected benefit of IFN-alpha in malignancies cannot be attributed to a single cause but rather to a chain of biological events non-reduceable to the same denomination (Table IV).

The most pronounced structural change seen in HCL following IFN-alpha administration (Figs 1, 2).

Following IFN-alpha administration not only externally visible changes — tending towards reverting to normal — are noticeable but both in the healthy and pathological lymphocytes there is a special histological change in the form of a tuboreticular inclusion within the subcellular organelles. This latter observation is, however, not in accordance with the literary findings where these changes are presumed to occur only in the cells affected by the disease (Fig. 3) /18/.

A clinician often encounters immunosuppressive sequelae of malignant diseases in the form of herpes zoster skin eruptions. The course of the

Table II

"Decreasing", "inhibiting" effects of IFN-α

Features
Blocking of DTH
Antagonism against PDGF
Blocking of growth increasing effect of IL-6
Decreasing of expression of IgE-FcR II. (?)
Decreasing particle numbers of CD_4 with HIV infection
Down regulation of oncogene expression
IFN-y could defend antiproliferative effect on neuroblastic cell-line
Decrease activity of DNA-polymerase

Table III

"Increasing", "stimulating" effects of IFN-α

Features
Stimulates thymocyte blast transformation
Stimulates formation of IFN-y
Increases concentration of IgE
Increases titre of progesterone receptor
Stimulates formation of IL-1 in GM-CSF induced macrophages
Increases titre of ICAM-1
Increases expression of CD_3, CD_8, CD_{23}, CD_{25} and CD_{56}
Increases titre of anti-HB_c Ag

Table IV

Effect of IFN-α in tumourous diseases

Features
Direct cytotoxic effect
Antiproliferative effect — by way tumour suppressor gene
Decrease tumour cell sensitivity against NK cells
Activation NK cell receptors through TAC antigens
Change pharmacokinetics of Alkeran
Activation of LAK cells
Synergism with cytotoxic effect of alkylating agents and Prednisolone

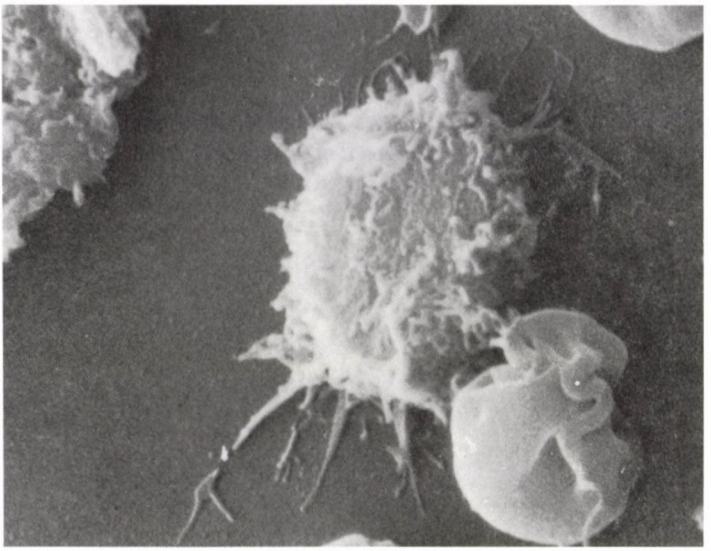

Fig. 1. Lymphocyte in HCL, prior to treatment

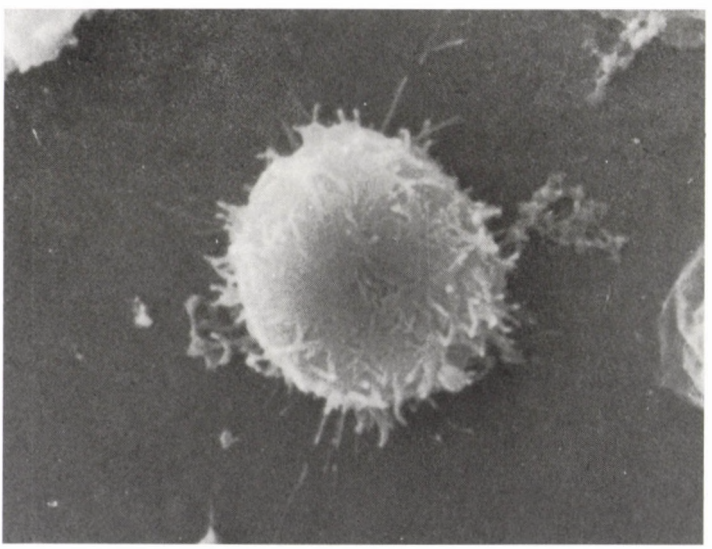

Fig. 2. Lymphocyte in HCL, after the treatment

disease cannot be influenced much by IFN-alpha therapy. It should be emphasized, however, that while given concomitantly with the skin eruptions, postherpetic root pains could be abolished and generalized spread of the lesions was prevented (Figs 4, 5).

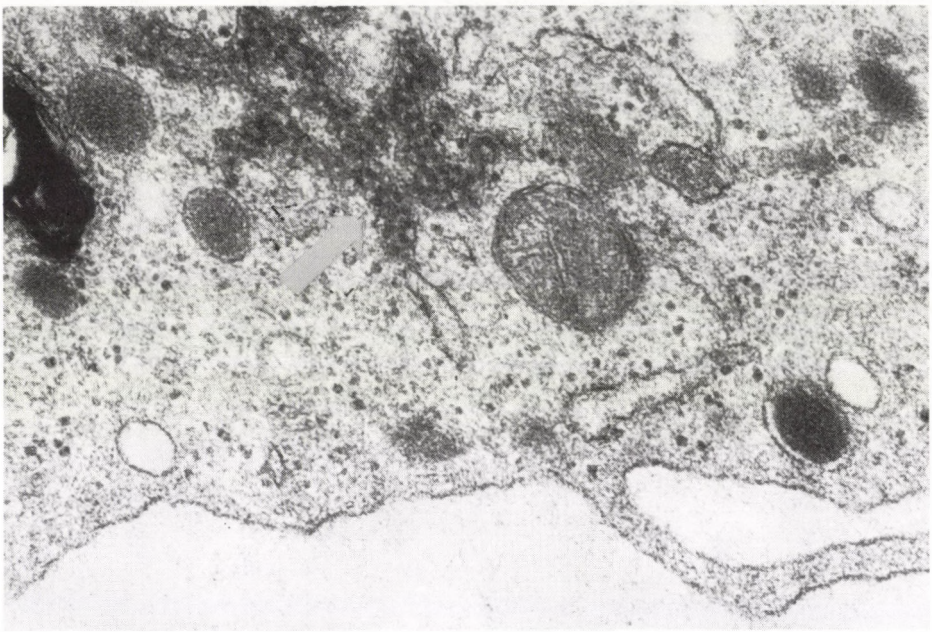

Fig. 3. Tuboreticular inclusions in the cytoplasm of lymphocyte

Based on our experiences we may say that a short term (1-2 months) IFN-alpha therapy will not lead to undesirable side effects apart from those mentioned above, whereas a long term, continuous IFN-alpha administration requires further observations, follow-up and is disputable from the following points of view.

1. In the presence of autoimmune disease markers the administration of IFN-alpha is risky as e.g. in C virus carrier CAH cases it may worsen an immune-mediated hepatocyte damage /30/. This possibility should be kept in mind with rise in the aminotransferase levels. Even the inflammatory exacerbation may be of such a high degree in patients with cirrhosis of the liver that ultimately a hepatic failure may develop /15, 26/.

Data from literature are controversial in this respect, since enzyme levels of patients infected with the delta-agent revert to normal following IFN-alpha administration. Although after discontinuation of the treatment a relapse may occur /5/.

2. IFN-alpha administration is contraindicated in patients with chronic active hepatitis (non-carriers) and autoimmune diseases in remission phase as this may lead to exacerbation of the process /39/.

Fig. 4. Herpes zoster faciei in floribus

Fig. 5. Herpes zoster in healing stadium

3. 1% of patients who have undergone IFN-alpha therapy have neutralizing antibodies, although, antibodies may be found even in cases with no prior history of treatment /49/. In clinical practice one should think of the presence of antibodies when loss of appetite and weakness disappear during the treatment. There are some publications presuming a 30-60% incidence /50/. The latest publications consider this high percentage to be false and differentiate between the immunogenicity of IFN-alpha-2a and

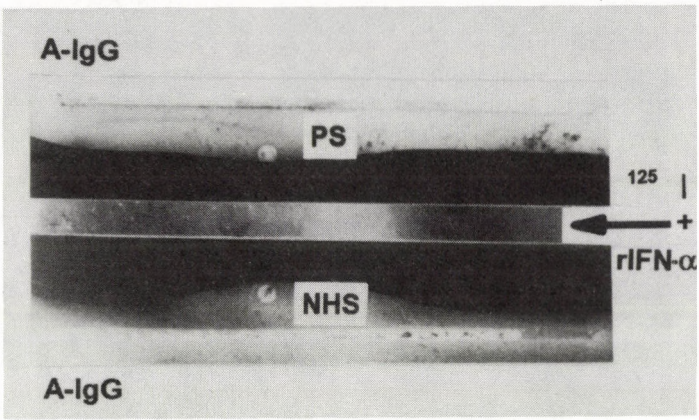

Fig. 6. Patient sera (PS) after the treatment of recombinant interferon-alpha, with antibody (IgG) against rIFN-alpha

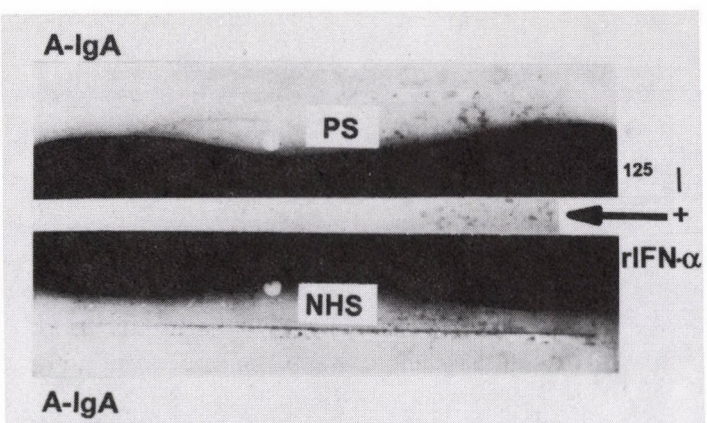

Fig. 7. Patient sera (PS) after the treatment of recombinant interferon-alpha, with antibody (IgA) against rIFN-alpha

IFN-alpha-2b. In the latter, the incidence is $< 3\%$ as seen from the results published in /43, 44/. We detected IgG and IgA type antibodies in HCL patients on long-term (4, 5 years) periodic treatment with our own autoradiographic technique (Figs 6, 7) /25/.

In the following, we summarize our present knowledge.

Production of antibodies capable of complex formation with IFN-alpha is dependent upon:

— the method and type of IFN production (biological or recombinant or its subtypes),

— the dose of IFN given,

— the time period of IFN treatment,

— the route of administration (subcutaneous, intracutaneous, intramuscular, etc.),

— the type of genetic background represented by the patient.

4. Upon follow-up of 69 patients with hairy cell leukaemia — diagnosed between 1983 and 1986 — we found that 13 of them had some type of malignancy 7.5 years later. Six cases out of the 13 developed a haematological malignancy /27/.

It has been proposed that the malignant change might be a result of the decreased immunity following IFN-alpha-2b treatment as well as of other direct effects of IFN-alpha-2b on cell proliferation and metabolism. These alternatives might be brought together even in a single process. IFN-alpha is able to block the production of growth factors involved in the autocrine regulation — at the same time enhancing the production of 2-5 A synthetase which, in turn, reduces the levels of TNF-alpha concentration affecting not only the cell metabolism of tumour cells but also that of the other cells and thus cells having a potential for malignant differentiation would be relieved of their control exerted by TNF-alpha /20/. One may not be able to exclude the above-mentioned detrimental effect of IFN-alpha on the chromosomes /21, 45/. Lastly, we should touch upon a practice which does not seem to decrease at all, namely that many papers have been published regarding IFN-alpha therapy, involving small groups of patients, having various diagnoses, using different doses for treatment getting varying results leading to various conclusions.

On analysis of these publications a question arises as to the cost/benefit ratio: whether it is permissible to spend huge amounts of money at such financially critical times on indications having no unequivocal support.

In summary: in spite of the many decades, experimental work and observations we are still unable to answer the following four basic questions claimed for by the outline of indications for therapy:

1. how much is to be given?
2. when should it be given?
3. the route of administration;
4. the time period of treatment.

(1) Intravenous administration of IFN-alpha will result in a 500-600 IU/ml peak level within 15-30 min. Its half life is 2 hours and the plasma IFN titer will revert to the basal level by the end of the 4th hour.

On intramuscular or subcutaneous administration peak levels will be reached in 4 h, and for the next 8-12 h a 100-200 IU/ml concentration will be recorded /19, 40/. A daily dose of 1 to 9 x 10^6 IU/dose of IFN-alpha, given intramuscularly, is fairly well tolerated in relation to adverse effects.

Severe toxicity is encountered with intramuscular administration at a daily dose of 10 to 18 x 10^6 IU/dose. Irreversible sequelae follow the daily administration of a 20 to 30 x 10^6 IU/dose. Irrespective of the indication a dose of 1 x 10^6 to 3 x 10^6 IU/day is given usually. The dose of recombinant IFN-alpha should be determined at 2- to 3-fold levels.

(2) Viral and malignant diseases should be managed differently regarding the initiation of treatment. In case of viral infections it should start as early as possible. The rationale behind this is to minimize the chances of developing complications and abolish the pain, e.g. in herpes zoster, as IFN bound to opiate receptors prevents pain /37/ as well as generalization of the disease process.

In our opinion the use of IFN-alpha in malignancies is justified only /17/ in conjunction with an induction therapy /27/ or in order to keep the disease "stationary", i.e. in remission, by administering it alone or in combination with steroids /7, 9/. Its use is not justifiable as part of a post-relapse combined treatment /10/.

(3) How should IFN-t be administered, considering its short half life, possible side effects and the characteristics of the preparations currently available in the market? These are given in the form of slow intravenous infusions /38/. A subcutaneous infusion (like the insulin pump) could prevent the side-effects affecting the central nervous system /14, 32/. In cases of long-term, repeated administrations, this is the most accepted route. For short-term, hopefully single use — the intramuscular route is preferred.

A few attempts have been made to give IFN-t directly into the lesion. It would be rather difficult to comment on these on the basis of literary data. Its combination with other drugs is still debated. There are no authentic data so far regarding the parameters determining the dose-ratio and the place of IFN in the combination (synergistic, adjuvant, potentiating, etc.). It is well known that IFN sensitizes cells to the effects of cytostatic agents concomintantly blocking the microsomal enzymes in the liver in vitro as well as in animal experiments. This fact should draw our attention to possible unwanted or expected drug interactions e.g. Placylyl (Abbott Laboratories Chicago) given together with IFN resulted in a reversible comatose state. Neurotoxic effect of Vinblastin is potentiated by IFN, Radiation therapy + IFN cause serious mucous membrane inflammation. We are far from solving these randomly brought up questions and cannot coming near to answer them by logic or experience. For the future, however, the following points should be kept in mind:

Very few anti-cancer drugs — if IFN could be regarded as an anti-tumour drug at all — would yield fast, clear-cut, well-analysable results upon their testing or in the early phase of treatment. There is no doubt that many years and decades have passed while tumour diagnostics emerged and developed. It is unfortunate to accept only prompt results, and not pay attention to the long-term, slow changes, their observations and registration.

It is debatable whether cytotoxicity studies of biological substances — in currently used systems — would yield reliable data and results. It has been noted that IFN treatment within given conditions involving diagnosis and stageing (under more or less standardized conditions) proved to be effective in one patient and ineffective in another. This observation leaves the question of sensitivity as well as the significance of research of the intracellular and membrane effects of IFN open. It should be stressed, however, that a resistance developing to IFN could be the result of propagation of another malignant clone and not the one for which the initial treatment — the first type of IFN was given.

Last but not least, the most important obstacle is that we lack the means of following the IFN-induced events. In spite of this "lack of knowledge" the future would be the combination of IFN-alpha with other cytokines.

(4) How long should the treatment last? Our answer would be based on clinical empiricism incorporating subjective rather than objective elements.

The goal should be reaching a remission phase. As a result of IFN-alpha therapy in malignancies complete remission (A) does not result in cure, the time of relapse is not much prolonged (B) the mean survival rate does not increase and at the same time the immediate and late adverse effects of therapy (C) cannot be neglected. Therapeutic use of IFN-alpha would lead to immediate (following the administration) and late adverse reactions. In lack of adequate information one should not comment on whether these symptoms are attributable to the effect, side effect or toxicity of the drug. However their registration is mandatory, so that all those who employ it would know /24/.

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Acta Medica Hungarica, Vol. 50/3—4, pp. 275—292 (1994)

CISPLATIN CONTAINING COMBINATION CHEMOTHERAPY OF ADVANCED GERM CELL LINE TESTICULAR TUMOURS

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(Received: September 23, 1994)

One hundred ninety patients with germ cell line testicular tumours were treated according to the modified Einhorn scheme. The response rate was 67.9%. The most favourable results were found in the embryonal histologic type (RR = 76.9%) in the biological markers (β-HCG and AFP) negative (RR = 97.4%) and in the minimal pulmonary extent group (RR = 94.1%). The authors treated 112 patients with including these VPB-resistant germ cell testicular tumour and those with recurrence after this treatment. The patients' mean age was 28.8 (limits 19 to 44) years. Patients were given Vepeside (100 mg/m in infusion for days 1-5), Adriablastin (40 mg/m in infusion on day 1) and Cisplatin (20 mg/m in infusion) for day 1-5. The treatment resulted in CR with 18 patients (16.1%) and PR with 42 (37.5%) (RR = 53.6%). The best results were obtained with the seminoma patients who were marker-negative and had small-volume metastasis. CR developed in 4 of 7 seminoma patients (57%) and in 7 of 25 marker-negative individuals (28%), and PR developed in 11 patients (44%) (RR = 72%). Out of 12 patients with small volume metastatis four (33%) showed CR and five revealed PR (41.7%), their RR turned out to be 74.6%. The average remission period was 37 (range 4-70) months in CR but merely 6.1 (range 2-38) months in PR. It can be stated that fairly good results can be achieved with second-line VpAP treatment in case of resistance developed to primary VPB therapy or subsequent relapse. The efficacy of combined chemotherapy of Vepesed + Holoxan +/- Adriablastin as third-choice was studied in advanced testicular cancer patients refractory to, or recurrent after, first- and second-line cytostatic therapy. Between September 1981 and January 1988 49 evaluable patients were treated with Vepesid (VP-16213 — 100 mg/m^2 days 1-5), Holoxan (40 ml/kg days 1-5), hydration, urine-alkylation + Uromitexan +/- Adriablastin (40 mg/m day 1). The single dose of Uromitexan was 20% of the daily dose of Holoxan, and the patients received it i.v. just prior to Holoxan administration (h 0), then 4 and 8 h later. Two patients got into CR and 10 to PR. The rate of remission was 24.5%. The most severe side effect was leukopenia. The elevation of BUN and se. creatinine was transient and mild. In those cases where Holoxan was not included in the first- or second-line regimens, when combined with Vepesid and Adriablastin as third-choice therapy one could achieve

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Abbreviations: CT = computer tomography, WBC = white blood cell, HCG = human chorion gonadotrophin, AFP = alpha-fetoprotein, RR = response or remission rate, adv. = advanced, abd. = abdominal, pulm. = pulmonary, hep. = hepatic, cereb. = cerebral, mediast. = mediastinal, tu. = tumour, supraclav. = supraclavicular, DDP = cisplatin, Vp = Vp-16213, Vepesid, Etoposid. ADM = adriamycin, adriblastin, BLM = bleomycin

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0236-5286/94/$ 4.00

further improvement. In case of CR the prolongation of life is also noteworthy. The first-, second- and third-line therapy plus salvage RLA and/or pulmonary metastasectomy achieved long-term survival only in one quarter of the patients.

Keywords: Advanced germ cell line, testicular cancer, cisplatin-containing chemotherapy

First line chemotherapy with vinblastine, bleomycin, cisplatin (VBP)

Testicular cancers do not belong to the tumours of high incidence, they total to merely 1 to 2% of all male malignancies. Their great significance is attributed to the fact that in the age group of 15 to 35 years they occupy the third place in the cancer mortality statistics, after leukemias and lymphomas.

Approximately 95% of the testicular tumours are of germ cell type, of them two thirds being nonseminomatous. While in Stage I and II seminomas radiotherapy is very successful, the nonseminomatous tumours show resistance, which explains the early choice of chemotherapy in this field. Li /10/ reported on noteworthy results obtained by the combination of actionomycin-D, methotrexate and chlorambucil. A great step forward was taken by Samuels et al. /19, 20/, whose publication on the combination of vinblastine (VBL) and bleomycin first appeared in 1973. The next advance was hall-marked by the team of Higby and Wallace, who performed a phase I study with cis-diamine-dichloroplatinum (cis-DDP) and found this drug effective in germinal neoplasms /10/, In 1974, Einhorn /3/ started investigations with VBP (VBL, BLM and cis-DDP), he published his excellent results in 1977. This is considered to be the beginning of the up-to-date combined cytostatic treatment of testicular tumours. Afterwards numerous teams /13, 14, 18, 20/ elaborated equally or similarly active combined cytostatic schemes (VAB-VI, PBVcr, BEP, VPBVe etc.). In Hungary, the National Institute of Oncology has made available to all our testicular cancer patients the platinum-containing combined cytostatic treatment since 1979. When we started our investigations, the results of VPB were the most favourable, in our Institute we apply the modified VPB as first-line therapy. In the last 5 years we have given a more advantageous BEP (Bleomycin, Etoposid and Cisplatin) schedule as a first-choice treatment for advanced disease. By the present time, this material has not been evaluated.

Patients and Methods

Between December 1979 and July 1986, 190 germ-cell-type nonseminomatous testicular cancer patients were treated. Their average age was 28 years ranging from 16 to 56 years. Histology was determined according to the WHO recommendation of 1981. Out of the 190 patients 78 had embryonal carcinomas, 1 yolk sac tumour, 13 teratomas, 6 choriocarcinomas and 92 mixed-type tumours. For restaging and measurements of the results physical examination, haemogram, 12-channel screening profile, serum beta subunit HCG, alpha-fetoprotein, chest X-ray, abdominal ultrasound and/or CT-scan were used. Brain, bone and liver isotopic- or CT-scan were performed when clinically warranted. Of the 190 patients 43 were in Stage II/B, 3 in III/A and 144 in III/B. The modified Einhorn scheme (VPB) was applied:

vinblastine	6 mg/m^2	i.v.	on days 1 and 2
cisplatin	20 mg/m^2	infusion	on days 1-5
bleomycin	30 mg	i.m.	on days 2, 9 and 16

The patients were pre- and posthydrated with 500 ml of 5% glucose, and cisplatin was administered in 500-1000 ml Rindex or physiological saline. The courses of treatment were repeated every 4 weeks. Patients progressing despite therapy after the second cycle were taken off the study and were given second-line (vepeside, adriablastin, cisplatin) treatment. The evaluation of response and toxicity was performed according to the as recommended by the WHO. Complete response (CR) means that the clinical symptoms, the radiological, abdominal ultrasound, CT, brain, liver and bone isotopic- or CT-scan lesions had completely regressed, and the increased levels of radioimmunoassay, alpha-fetoprotein and beta-HCG serum observed prior to the start of treatment fully normalized. Partial response (PR) was considered to be a 50% or more decrease in the diameter of measurable lesions. A 25% or greater increase in the diameter of measurable lesions was regarded as progression (Porg). Between PR and Prog there were patients with no response (NR).

Results

Of the 190 patients, 70 (36.8%) showed CR and 59 (31.1%) showed PR. The total response rate (CR + PR, i.e. RR) was 67.9%. The correlation between histology and response is shown in Table I. Out of 78 patients with embryonal carcinoma, CR was achieved in 32 (41.0%). PR was observed in 28 patients (38.9%). The respective figures for the 6 NR and 12 Prog patients are 7.7% and 15.4%. The yolk sac tumour group was not evaluable, due to the low number of cases. Thirteen patients belonged to the teratoma histological group, 1 of them (7.7%) achieved CR: in 4 cases PR (30.8%), in 3 cases NR (23.1%), and in 5 cases Prog (38.5%) was observed. In the choriocarcinoma group only 1 of the 6 (16.7%) patients developed CR. PR was seen in 2 cases (33.3%). Half of the choriocarcinoma patients failed to respond to the therapy, they had a very high liter of beta-HCG and were in a far advanced stage. In the group of mixed type tumours 35 patients of the total 92 (38%) achieved CR, 26 (28.2%) had PR, in 15 (16.3%) NR, and in 17 (18.5%) Prog was seen.

Table I

Correlation between histology and response

Histology	CR	PR	NR	PROG	Total No.
Embryonal carcinoma	32	28	6	12	78
Yolk sac tumour	1				1
Teratoma	1	4	3	5	13
Choriocarcinoma	1	2		3	6
Mixed type	35	25	15	17	92
Total	70	59	24	37	190

Table II

Correlation between biological markers and response

Biological marker	CR	PR	NR	Prog	Total No.
Elevated AFP	27 44%	12 20%	9 15%	13 21%	61
Elevated HCG	7 26%	9 33%	3 11%	8 30%	27
Elevated AFP+HCG	13 21%	23 36%	12 19%	15 24%	63
Normal AFP+HCG	23 59%	15 38%		1 2%	39
Total	70	59	24	37	190

When comparing the different groups, we found RR (CR + PR) in 76.9% in the embryonal carcinoma group, 38.5% in teratoma, 50% in choriocarcinoma and 65.4% in the mixed type group. With respect to remission, embryonal carcinoma proved to be the most and teratoma the least, favourable.

The response according to histological markers is shown in Table II.

The response in function of the extent of disease was also studied: the results are shown on Table III.

The most frequent side effects were nausea, vomiting and leukopenia. Generally, leukopenia was the most severe side effect. WBC was below 1000 in 7% of the cycles. Gastrointestinal toxicity occurred in severe, moderate and mild forms. Nephrotoxicty appeared in 4% of cycles, with an increase in the level of blood urea nitrogen and serum-creatinine. This phenomenon, however, was of moderate degree and transient, and could be treated by hydration.

Table III

Correlation between extent of disease and response

Histology	CR	PR	NR	PROG	Total No.
Min. pulm.	30	2	2	—	34
	88%	5%	6%	—	
Adv. pulm.	17	15	6	8	46
	37%	33%	13%	17%	—
Abd.+supraclav. and/or mediast	—	2	—	1	3
Min. pulm.+min. abd.	5	2	—		7
Min. pulm.+adv. abd.	3	8	1	6	18
	17%	44%	6%	33%	—
Adv. pulm.+adv. abd.	6	19	4	8	37
	16%	51%	11%	22%	—
Adv. abd.	8	11	11	13	43
	18%	26%	26%	30%	
Abd.+liver	1			1	2
Total	70	59	24	37	190

Following treatment, after the 2nd or 3rd cycle, in 58% of the patients considerable hair loss was seen. As a side effect of bleomycin hyperkeratosis and desquamation were observed only in 5 cases: pulmonary fibrosis was not seen. As regards VBL, intestinal paralytic symptoms occurred in 6.8% (13 patients), which responded well to parasympathomimetic agents.

Discussion

The VPB cytostatic combination is one of the most widespread approaches in the treatment of germ cell testicular tumours. The advantages of this therapy are high CR and RR, except for the far advanced and bulky tumours. No expensive instruments (e.g. continuous delivery drug pump) are necessary, the performance of the treatment is simple, the myelosuppressive effects are considerable. In 1977, Einhorn et al. /3/ were the first to publish results obtained with the VPB combination. Out of 47 evaluable cases, 35 CR (74%) and 12 PR (26%) were achieved (RR = 100%). The rate of CR after the surgical removal of the residual tumour was 81%, in the case of minimal abdominal and pulmonary disease 88%, in advanced pulmonary and ab-

dominal disease 67% and 56%. In 1980 Williams and Einhorn et al. /30/ reported on a larger group of patients. They applied the VPB treatment to 78 patients and achieved CR in 53 (63%) and PR in 23 (30%) patients. After the surgical removal of the residual tumour CR was seen in 82% while the RR was 98%. It was confirmed again that the patients with advanced pulmonary and abdominal metastases were worse responders than those having minimal metastases. CR in the pulmonary metastatic group was 50%, in the abdominal group it was 43%.

Bosl /1/ reported that in 20 out of 28 patients with metastatic testicular cancer chemotherapy was the only treatment that resulted in CR (71%). In 3 patients the residual tumours, following surgery, proved to be necrosis or fibrosis, therefore, these were also regarded as CR, thus 82% of the cases showed CR.

The SWOG (Southwest Oncology Group) /6/ described VPB therapeutic results of 135 patients, in 82 patients CR (61%), in 32 PR patients (24%) was seen (RR = 85%). In 1984 the SWOG reported from another study including 110 patients with the following results: CR 60 patients (53%), PR 42 patients (37%), RR 90%.

We had an unselected group of 190 patients of whom 41 had minimal pulmonary or pulmonary + abdominal metastases. This is likely to explain the lower RR of the whole group. The most favourable results approaching the best results described in the literature were seen in the minimal pulmonary and marker-negative cases. Four-fifths of our patients were in a far advanced stage, which understandably influenced the total parameters of the entire group.

The VPB combination is a very effective treatment for disseminated testicular cancer, but due to its high toxicity, the patients should be controlled most carefully.

Second-line chemotherapy of VBP resistant metastatic testicular cancer

As a rule, 30-40% of advanced tumour patients require — even according to the best statistical results — continual therapy for progression was noted at first admission or for a relapse developed later on. With stage I/B or stage II patients undergone retroperitoneal lymphadenectomy (RLA) combined with adjuvant Cisplatin therapy one has to reckon with a relapse in

Table IV

Second-line therapy of VPB-resistant patients with Vepesid combined with Cisplatin

Authors	Combinations	Pts. No.	CR No.	PR No.	RR (%)
Williams et al. /30/	Vp+DDP+/-BLM	8	5	3	100
	VP+DDP+ADM+/-BLM	20	9	9	90
Vogelzang, Kennedy /24/	Vp+DDP+ADM+BLM	7	4	4	100
	Vp+DDP+ADM	11	1	10	100
Bosl /1/	Vp+DDP	19	0	0	0
Lederman et al. /9/	Vp+DDP	12	5	4	75
Haisworth et al. /6/	Vp+DDP+/-ADM+/-BLM	45	19	12	70
Hansen et al. /7/	Vp+DDP+BLM	26	6	11	65
Total		158	48	53	64

10 to 15% of the cases. This observation speaks in favour of continuing their therapy. Without further effective treatment these patients survive not longer than for severe months /6, 15/. The promising therapeutic potentials of current chemotherapy and demands of clinical practice all necessitate the development and introduction of new, more potent cytostatics.

Positive clinical achievements were noted in the therapy of the above tumours with Vepeside (VP-16213 or Etoposide). Even the VPB resistant non-seminoma type germ cell testicular cancers were responsive to Vepeside in phase I and II studies /5, 12, 30/.

In animal experiments Schabel et al. /21/ demonstrated in 1979 synergic therapeutic effects and enhanced additive toxicity of Vepeside and Cisplatin.

Almost simultaneously, studies were started with Vepeside in combined second-line therapy of VPB resistant patients (Table IV).

Clinical results have confirmed the synergic effect of Vepeside and Cisplatin; toxicity has also proved to be additive.

Patients and Methods

Altogether 112 VPB resistant patients or those developing relapse after this therapy were treated by us during the period from October 1983 to January 1987. The patients' mean age was 28.8 years (range: 19 to 44 years). Distribution of patients according to tumour type: no

detectable tumour in spite of high AFP value (1 patient); inoperable II/B, III/A (19 patients) and III/B stage tumours (89 patients); seminoma (4 patients); embryonal carcinoma (47 patients); teratoma (6 patients); choriocarcinoma (4 patients); endodermal sinus tumour (1 patient); germinal tumour of mixed type (47 patients). In 30 patients elevated AFP level and in 20 others high beta-HCG level was found. In 37 cases, however, both markers were elevated, but in 25 cases, they were found within the physiological range. As a first-line therapy all the 112 patients received VPB, two of them were given radiotherapy, too before chemotherapy. At our Department the second-line chemotherapy was conducted according to the following schedule:

Vepeside	100 mg/m^2	in infusion	days 1-5
Adriablastin	40 mg/m^2	in infusion	day 1
Cisplatin	20 mg/m^2	in infusion	days 1-5

If laboratory parameters permitted, this therapy was repeated at 4-week intervals. Patients receiving minimum 2 cycles were evaluated from the therapeutic point of view. Evaluation was made according to the international standards.

Results

The Vepeside + Adriablastin + Cisplatinum (VpAP) combination as a second-line therapy was applied to 112 OVB resistant germ cell testicular cancer patients.

CR was achieved in 18 patients (16.1%) and PR in 42 (37.5%). In the evaluation of testicular cancers we regard only CR and PR as a therapeutic effect, their summarized value expresses the remission rate (RR) that in our case was 53.6%. Twenty-five patients did not respond to the therapy (22.3%) and with 27 patients (24.1%) progression was observed.

Therapeutic results were further analysed in function of histological diagnosis, biological markers and tumour extension. The relationship between histological structure and remission is demonstrated in Table V.

Table V makes it clear that patients occurred in higher number only in the groups of embryonal carcinoma and mixed tumour. Therefore, we could only calculate remission rate only for these groups, they were 48.9% and 63.8%, respectively. If the seminoma groups and the anaplastic seminoma groups are compared to each-other, it is conspicous that in 4/7 patients (57%) CR could be achieved with second-line therapy, too. In the non-seminoma groups, however, in cases of statistically evaluable embryonal carcinoma, only 6/47 patients (12.76%) showed CR. With patients having mixed type tumour CR was observed in 7/47 cases (14.89%).

Because of different features therapeutic results relating to the other groups cannot be summarized, and the low case number would not permit to draw an established conclusion. However, CR achieved in the choriocarcinoma group with very poor prognosis in noteworthy, indeed. The correlation between bilogical markers and remission was also studied (Table VI).

Table V

Correlation between histology and remission

Histology	CR	PR	NR	PROG	Patients No.	RR (%)
Seminoma	2	—	1	—	3	
Anaplastic seminoma	2	—	1	1	4	
Embryonal carcinoma	6	17	12	12	47	48.90
Yolk sac tumour	—	—	1	—	1	
Teratoma	—	2	3	1	6	
Choriocarcinoma	1	—	—	3	4	
Mixed tumours	7	23	7	10	47	63.8
Total	18	42	25	27	112	

Table VI

Correlation between biological markers and remission

Biological markers	CR	PR	NR	PROG	Patients No.	RR (%)
Elevated AFP	5	11	7	7	30	53.30
Elevated HCG	2	9	4	5	20	55.00
Elevated AFP+HCG	4	11	9	13	37	40.50
Normal AFP+HCG	7	11	5	2	25	72.00
Total	18	42	25	27	112	

Analysis of these data makes it unequivocal that the best therapeutic effectiveness was observed with those having normal biological markers (AFP + HCG), in their group the RR was 72%. Of course, highest CR value was found also in this group, this meant 7/25 cases (28%). RR values in the groups of high AFP and high HCG levels were almost identical; 53.3% and 55%, respectively. In the group of high AFP level CR could be demonstrated in 5/30 patients (16.66%) in contrast to the high HCG group where CR was identified only in 2/20 patients (10%). The lowest remission rate (40.5%) was associated by the elevation of both markers, but only 4/37 patients (10.8%) revealed CR. The relationship between disease extension and remission is represented in Table VII.

In spite of the considerable number of patients, only four of the 11 groups included more than 10 patients. For these four groups the RR values

Table VII

Relationship between disease extension and remission

Disease extension	CR	PR	NR	PROG	Patients No.	RR (%)
High AFP only	1	—	—	—	1	
Min. pulm.	3	1	—	—	4	
Adv. pulm.	5	6	4	2	15	60.00
Min. abd.	—	1	3	—	4	
Adv. abd.	3	7	2	6	18	55.60
Min. pulm.+min. abd.	—	3	—	—	3	
Min. pulm.+adv. abd.	2	4	2	3	11	54.50
Adv. pulm.+min. abd.	1	1	—	1	3	
Adv. pulm.+adv. abd.	3	18	13	12	46	45.70
Adv. abd. and/or Med. Sup.	1	1	—	2	4	
Adv. abd.+adv. pulm. + + other organ	1	—	1	1	3	
Total	18	42	25	27	112	

Table VIII

Side effects of VpAP treatment

Side effects	WHO grades (%)				Total (%)
	1	2	3	4	
Vomiting, nausea	52.00	38.30	3.50	—	93.70
Leukopenia	—	50.90	15.20	8.00	74.10
Nephrotoxicity	3.60	—	—	—	3.60
Hepatotoxicity	—	2.70	—	—	2.70
Cardiotoxicity	2.70	—	—	—	2.70
Alopecia	—	25.00	—	—	100.00

were also indicated. Of them 46 patients belonging to the group of M. pulm. + M. abd. involvement had reached a statistically evaluable level. In this highly advanced stage with marked extension, CR was observed with as few as 3 patients (6.5%). When examining the few patients with slight tumour extension or metastasis together with those of positive AFP finding (high AFP; S. pulm., S. abd., S. pulm. + slight abd.) we had 12 patients of whom 4 (33%) showed CR and 5 (41.6%) showed PR (RR = 74.6%). Out of 100 patients with large volume metastases 14 (14%) revealed CR and 37 showed PR (37%). Their

summarized RR was 52%. The x^2 test yielded $P < 0.10$ for CR and $P < 0.05$ for RR (merely a value of 0.2 was missing to reach the level of significance).

The average duration of was 37 (4-70) months for complete remission and it was 6.1 (2-38) months for partial remission.

Description of the therapeutic effectiveness of this aggressive therapy has to be complemented with its potential side effects (Table VIII).

The most common side effects were: nausea, vomiting, alopecia and leukopenia. Leukopenia under 1000 was the most dangerous consequence, it occurred in 8% of the cycles. Nadir was between days 9 and 11 calculated, from the first day of treatment. Nephrotoxicity manifested in temporary rise of BUN and/or se. creatinine levels that could be normalized with hydration in a few days. Only 3 patients had signs of cardiotoxicity; they complained of transient ES. Hepatotoxicity was shown as elevated liver enzyme and/or se. bilirubin values, signs which normalized under conservative therapy (diet and Reducdyn) in a few weeks.

Discussion

Favourable experience has been obtained with the second-line VpAP combined therapy of 112 VPB resistant testicular cancer patients. In contrast to the 67.89% remission rate of the first line VPB therapy the remission rate of second-line VpAP therapy was 53.7%, which is a fairly good results. During primary VPB therapy the CR value was 36.8%. After second-line therapy it decreases drastically to 16.1%. The lower RR values and particularly the CR ones during second-line therapy were naturally due to the fact that cells sensitive to chemotherapy had died during primary treatment and thus the second-line therapy acted against a much more resistant tumour cell line. Signs suggestive of more favourable prognosis, like the negative markers or small size metastases are valid in second line therapy, too. In many institutes, the second-line therapy usually consists of Vepeside + Cisplatin. Lederman /8/, however, think that combination of Vepeside + Adriblastin + Cisplatin to be more potent. We chose this latter combination because in the early 80s we succeeded in achieving CR in some VPB resistant patients using the combined administration of Adriblastin and Cisplatin although not so many times as with the combination containing additional Vepeside. Others' investigations mentioned in the foregoing and our own observations confirm the fact that VpAP is a potent combination that may open new vistas in second-line therapy.

Third-line chemotherapy of resistant advanced testicular cancer

In the minority of advanced germ-cell testicular cancers the above first-choice treatments are not effective, the disease further progresses and almost 15% of CR patients develop recurrences. Relapse may occur after stage-II adjuvant treatments as well. In most parts of the world, treatment regimes containing Vepeside (VP-16213) and cisplatin /1, 6, 9, 16, 30/ are widely used as a second-choice. Literature data and our own observation suggest that in 24 to 43% of the patients complete remission (CR) can be achieved. The results of high-dose chemotherapy (usually Vepeside and/or Cisplatin), with or without bone marrow transplantations, show that despite the higher rate of complete remissions the survival time is not prolonged /3/.

Patients refractory to the previously described therapies or relapsing after second-choice treatment need further treatment. On the other hand, we can read about the less favourable results of third-choice therapies.

It seems that in those cases where Holoxan (Ifosfamide) is not included in the primary or secondary treatment it may be an important component of the third-choice therapy. Ifosfamide was proved to be an effective drug by Weissbach /27, 28/ and Schmoll /22, 23/.

In consideration of all above we tried to apply the combination of Vepesid (VP-16213, Etoposide) + Adriablastin + Holoxan as third-choice treatment.

Patients and Methods

Between September 1981 and January 1988, 49 patients resistant to primary and secondary treatment or those with recurrent disease were studied.

The median age of patients was 28.66 years, ranging from 17 to 66. In 2 patients retroperitoneal lymphadenectomy (RLA), in 9 patients salvage RLA or debulking surgery were performed, 2 patients with seminoma received radiotherapy as first-line treatment.

Two patients had seminoma, 2 anaplastic seminoma, 18 had embryonal carcinoma, 7 had teratoma and 20 suffered from mixed type germ cell testicular tumour. Three patients had inoperable bulky abdominal i.e. stage II/B disease, 2 patients were in clinical stage III/A and 44 in stage III/B.

All patients received 4 to 6 cycles of VBP as first-line therapy, thereafter 2 to 6 cycles of VAP (Vepesid, Adriablastin, Cisplatin) as second-line treatment. Then the following third-line scheme was used:

Vepesid	100 mg/m^2	in infusion	on days 1-5
Holoxan	40 mg/kg	in infusion	on days 1-5
alkylation, hydration, Uromitexan			
Adriablastin	40 mg/m^2	in infusion	on day 1

Holoxan was administered in 1000 ml Rindex, prae- and posthydration were applied in the form of 1500 to 2000 ml glucose or physiologic saline. After Holoxan administration the urine pH was kept about 7, either by i.v. sodium bicarbonate solution or peros alkaline mineral water. The dose of Uromitexan correspond to 20% of the daily dose of Holoxan and was applied i.v. prior to, and 4 and 8 after, Holoxan administration. Microscopic study of the urinary sediment was performed prior to therapy, on days 3 and 6, in case of microscopic haematuria every 2nd or 3rd day until elimination of the sediment.

Results

The response was evaluated after the second treatment cycle.

Two patients showed complete remission, 10 patients partial remission, the rate of remission was 24.5%. Moderate remission (MR) was seen in 7 patients, stable disease (SD) in 9, progression (PROG) despite therapy in 21 patients.

In the 2 patients with seminoma and 2 with anaplastic seminoma no considerable effects were observed, 1 patient had stable disease and in the other cases progression took place. Due to the earlier irradiation, to diminish toxicity, 25% dose reduction was necessary. CR was seen in 2 of 18 patients with embryonal carcinoma, 5 PR, 4 MR, 3 SD and 4 PROG were observed. The most favourable response, 38.9% was RR, was seen also in this group. Of the 7 patients with teratoma 1 PR, 2 MR, 2 SD and 2 PROG were recorded. Although the majority of the patients with mixed type tumours showed progression (60%), 4 PR, 2 MR and 2 SD were also observed.

Although the number of patients in the individual groups is small to detect statistically significant difference, it seems evident that CR was achieved in a supraclavicular and/or mediastinal and in an abdominal + pulmonary case. In the first 4 groups of Table XI, there were 32 patients, none of whom had cerebral or hepatic metastasis. In this group 2 and 6 patients showed CR and PR, respectively. Seventeen patients belonged to the other 4 groups, all of them had cerebral, hepatic or both types of metastases, and only 4 patients developed PR.

Sixteen patients had elevated AFP serum level, in 1 case CR, in 2 cases PR, in 4 cases MR, in 4 cases SD and in 5 cases progression was observed. In 11 patients high serum β-HCG was found, in 1 of them CR, in 2 PR, in 1 MR and in 6 patients progression was detected. Elevated AFP + β-HCG was recorded in 16 patients, the response in this group was as follows: 4 PR, 2 MR, 2 SD and 8 PROG. The AFP + β-HCG level was not elevated in 6 patients, 2 of them had PR, 2 SD and 2 PROG.

Table IX

Therapeutic results

Number of patients	CR	PR	MR	SD	PROG
49	2 4.1%	10 20.4%	7 14.3%	9 18.4%	21 42.9%

Table X

Correlation between histology and remission

Histology	CR	PR	MR	SD	PROG
Seminoma				1	12
Anaplastic seminoma					2
Embryonal carcinoma	2	5	4	3	4
Teratoma		1	1	2	2
Mixed type tumour		4	2	2	12
Total	2	10	7	9	21

Table XI

Correlation between disease extension and remission

Site of metastasis	CR	PR	MR	SD	PROG
Supracav. and/or mediast.	1	1			
Adv. pulm.		3	2		4
Adv. abd.					2
Pulm. and abd.	1	2	3	7	6
Adv. pulm. and cereb.		2			1
Adv. abd. and cereb.		1	1	1	3
Adv. abd., hep., pulm.		1	1	1	4
Adv. abd., hep., pulm., cereb.			1		1
Total	2	10	7	9	21

The most common side effects were alopecia, leukopenia, nausea and vomiting. Alopecia occurred already after the first treatment cycle, between days 20 and 30 after the initiation of therapy. The most severe side effect was leukopenia, the WBC count was below 1000/µliter in 17% of the cycles.

Table XII

Correlation between biological markers and remission

Biological marker	CR	PR	MR	SD	PROG
Elevated AFP	1	2	4	4	5
Elevated beta-HCG	1	2	1	1	6
Elevated AFP+beta-HCG		4	2	2	8
Normal AFP+beta-HCG		2		2	2
Total	2	10	7	9	21

Table XIII

Toxicity

Toxicity	Total (%)	WHO grade			
		1	2	3	4
		% of patients			
Nausea and vomiting	40.0	4.0	34.50	1.5	
Leukopenia	52.0	21.0	5.0	9.0	17.0
Thrombopenia	14.50		1.50	5.0	8.0
Alopecia	100.0		17.0	83.0	
Haematuria	6.0	6.0			
Elevated se. creatinine	3.80	3.80			
Elevated BUN	1.5	1.50			

The nadir was only day 8 (range days 3 to 15). Thrombopenia generally associated with leukopenia, occurred in 14%. Nausea and vomiting mainly moderate, which tended to be eased by antiemetics, were registered in 40% of the cycles. Microhaematuria developed in 6% of the cycles, but macrohaematuria occurred in none of the cases. Mild and transient elevation of BUN and se. creatinine was observed in 1.5%, and 3.8%, respectively, but these levels fell to the normal within a few days by continuous hydration.

Discussion

The prognosis of patients refractory to or recurrent after primary or secondary treatment is very poor, in the majority of cases further chemo-

therapy is necessary. Each institution is determined to apply some individual solutions, but in such incurable cases one can hardly achieve any result. This is why the literature lacks publication on this topic.

In the part two years Einhorn et al. /3/ published papers on the VIP (VP-16, Ifosfamide, Cisplatin) treatment used as third-choice approach. They found a RR in 37% out of which 21% were CR. Between September 1981 and January 1988 the same authors treated 49 evaluable patients with the combination of Vepesid + Holoxan +/- Adriablastin. (Fifteen patients did not receive Adriablastin in the course of the third choice therapy.) They reported twice on their results /5, 30/. Most of the patients suffered from far-advanced disease. Out of them 17 had cerebral and/or hepatic metastasis in addition to the pulmonary and abdominal ones. The authors failed to reproduce the results obtained by Einhorn et al., only 24.8% RR was reported. Only 2 patients achieved CR, which lasted for 36 months in one of the patient and 17 months in the. In the former case, prior to treatment extensive abdominal or pulmonary, in the latter only supraclavicular and mediastinal metastases were detected. Ten patients showed PR, with a median duration of 4.5 months (range from 1 to 6.5). The best remission rate was observed in the embryonal carcinoma. The poorest results were seen in patients with the elevated β-HCG and AFP + β-HCG groups with 66% and 50% of progression, respectively. Since 1989, we have applied VIP (Vepesid + Ifosfamide + Cisplatin) combination as third-line therapy, but it has not been evaluated by the present time.

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Acta Medica Hungarica, Vol. 50/3—4, pp. 293—294 (1994)

BOOK REVIEW

PROGRESS IN DRUG RESEARCH, Vol. 43.

Ed.: Ernst Jucker

Basle, Switzerland, 1994

PDR Vol. 43 consists of five extensive survey articles of pharmaceutical research. The five topics of the current issue are:

1. Cholesterol-lowering drugs and their effectiveness in the practice
2. Antihypertensive drugs in the past 50 years
3. Natural polymaines and their role in the immune system
4. Summary of biologially active quinazolones
5. Interferons — gene regulation and expression

All five topics of the current issue are reviewed by widely-recognized experts. The issue is highly recommended for practitioners dealing with hypertension in the everyday health-care (topic 2), and clinicians specialized in metabolic disorders (hyperlipidaemia, topic 1). The other three topics are useful for specialists and researchers working in the field of experimental immunology (topics 3 and 5) and topics 4. extensive cousists and review of quinazolones used in human therapy.

Issue 43, 1994 consists of 336 pages including index for vol. 43. Price SF 298.-

SUMMARIES AND COMMENTS

Topic 1. Drugs for treatment of patients with high cholesterol levels and other dyslipidaemias. Bays, H. E. and Dujovene, C. (32 pages including 101 references and 13 tables)

Summary: Recommendations for artherosclerotic coronary artery disease (ASCAD) therapy should begin with evaluation and treatment of the underlying conditions that may contribute to hyperlipidaemia, use of possible alternatives to drugs that may worsen dyslipaemia, encouragement for a healthy life style diet and physical exercise. If dyslipaemia persists, the most cost-effective approach towards recommending the most appropriate lipid-acting drug is to reserved for treatment of patients with, or at high risk for, ASCAD whose expected benefit outweights the potential risks and excessive costs.

Comments: The author has succeeded in keeping in balance between the growing amount of information to cite and the amount of important message to deliver. His conclusions are acceptable. He does not over-emphasize any of the drugs and studies. All clinicians should read and keep in mind his message.

Topic 2. In search of ideal antihypertensive drugs: Progress in five decades. Lien, E. J., Gao, H. and Lien, L. L. (44 pages, including 6 tables and 139 references)

Summary and comments: The author reviews all groups of antihypertensive drugs used in the past 50 years. The review serves as a short catalogue groups of drugs, listing the structure activity relationship, biopharmaceutics and pharmacokinetics, pharmacology and indication, dosage, adverse

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reactions and interactions with other drugs. The work is impressive and important in the everyday practice. This review may serve also students studying antihypertensive treatment and clinicians who are not familiar with new families of antihypertensive drugs.

Topic 3. The natural polymaines and the immune system. Seiler, N. and Atanassov, C. L. (56 pages, including 254 references and 8 figures)

Summary: Natural polyamines, such as putrescine, spermidine and spermine are in may ways involved in host defense mechanisms. They participate in macromolecular syntheses, in the structural organization of numerous cell components, and they modulate and regulate enzyme activites. The difficulty of pinpoint polyamine functions is due to their low importance within cellular machinery. All known functions of polyamines are reviewed in an extensive manner. This review recommended for researchers working in field of cellular immunology and biology.

Topic 4. Biologically active quinazolones. Sinha, S. and Srivastava, M. (96 pages, including 374 references and 12 additional pages as addendum)

Summary and comments: This extensive review lists 216 quinazolones, presenting their chemical structures and known biological activites. The new quinazolones of the last 13 years are dealth with. (The lastest review one the same topic was published by Johne S. in 1981 in PDR.) The large body of data proves the importance and effectiveness of quinazolones from antihelmintic therapy to hypoglycaemic activity. This article is suggested for specialists for quinazolone research.

Topic 5. Production and action of interferons: New insights into molecular mechanisms of gene regulation and expression. Hayes, M. P. and Zoon, K. C. (32 pages, including 211 references)

Summary and comments: New information about interferon (IFN) regulation and IFN-coding genes opened a new insights into intracellular signalling. The IFN system has provided a model system for the study of cytokines and the regulation of gene expression. Understanding of the stringent controls governing the production and action of cytokines makes us able to utilize these agents in the clinic effectively. This article is useful for researchers engaged in the molecular biology and for immunologists in basic research. At last it presents an updated summary for clinical immunologists about the gene regulation and basic principles of interferon biology.

Z. TULASSAY, M.D.

Acta Medica Hungarica, Vol. 50/3—4, pp. 295—296 (1994)

FAREWELL TO ACTA MEDICA HUNGARICA

THE READER HOLDS THE LAST ISSUE OF THIS PERIODICAL IN HIS HANDS, A VERY SAD ANNOUNCEMENT BY THE EDITOR-IN-CHIEF. OUR ACTA MEDICA HAS COME TO AN END WITH ITS VOLUME 50.

ACTA MEDICA, LIKE OTHER PERIODICALS WRITTEN IN FOREIGN LANGUAGES, WAS FOUNDED BY THE HUNGARIAN ACADEMY OF SCIENCES SOON AFTER WORLD WAR II, WHEN OUR DEVASTATED COUNTRY WAS SLOWLY RECOVERING. IT STARTED AT A HISTORICAL MOMENT WHEN THE DOWNROLLING IRON CURTAIN THREATENED OUR SCIENTIFIC SCHOOLS FROM BEING COMPLETELY ISOLATED FROM ABROAD. OUR JOURNAL WAS AIMED AT ALLOWING THE FREE FLOW OF INFORMATION IN SPITE OF A STRONG COUNTERPRESSURE. THUS, A WINDOW OF VITAL IMPORTANCE WAS OPENED TOWARDS THE WORLD OF INTERNATIONAL SCIENCE.

ALL HUNGARIAN PHYSICIANS WERE AWARE OF THIS FACT, AND SO WERE THE EDITORS OF THIS JOURNAL. IN THE FOLLOWING YEARS BOTH THE AUTHORS AND THE EDITORS MADE EFFORTS TO PUBLISH THE BEST RESULTS OF THE HUNGARIAN MEDICAL RESEARCH IN ACTA MEDICA. AS A RESULT CRITICALLY REVIEWED, WELL-EDITED AND WELL-PRESENTED PUBLICATIONS WRITTEN IN ENGLISH AND OTHER LANGUAGES OF WIDE DISSEMINATION APPEARED IN IT.

OUR EFFORTS SOON BROUGHT ABOUT AN INTERNATIONAL ACKNOWLEDGEMENT OF ACTA MEDICA.

OF COURSE, OUR JOURNAL ENCOUNTERED DIFFICULTIES FROM THE VERY BEGINNING. PUBLICATION OF PAPERS IN FOUR LANGUAGES CAUSED PROBLEMS IN EDITING AND DISTRIBUTION; DUE TO DIFFICULTIES IN OBTAINING PAPER AND PRINTING FACILITIES, THE PUBLICATION OF THE ISSUES WERE OFTEN DELAYED, AND THE DISTRIBUTOR ALSO ENCOUNTERED DIFFICULTIES IN DELIVERING THEM ON TIME.

THE SITUATION CHANGED IN THE EARLY '80'S, WHEN HUNGARIAN SCIENTISTS WERE ALLOWED TO PUBLISH IN TRADITIONAL INTERNATIONAL JOURNALS, CONSEQUENTLY, WE RECEIVED GOOD-QUALITY MANUSCRIPTS IN AN EVER DECEASING NUMBER. THE DECLINING STANDARDS AND THE IRREGULAR APPEARANCE OF THE JOURNAL LED TO DELETION FROM CURRENT CONTENTS LIFE SCIENCES AND A CONSEQUENT LOSS OF

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IMPACT FACTOR. UNDER THE CHANGED CIRCUMSTANCES HUNGARIAN SCIENTISTS PREFER TO PUBLISH IN WELL-KNOWN TRADITIONAL PERIODICALS WITH HIGH IMPACT FACTORS.

AS EDITOR-IN-CHIEF I KNOW WELL THAT THE EXISTENCE OF ACTA MEDICA WAS NOT WITHOUT SERVICE TO SCIENCE. IT HELPED TO MAKE FOREIGN READERS FAMILIAR WITH THE WORK OF HUNGARIAN SCIENTISTS IN AN ERA WHEN THERE WAS NO OTHER WAY OPEN FOR THEM. THUS, IT CONTRIBUTED TO THE SURVIVAL AND FLOURISHING OF HUNGARIAN MEDICINE: THIS SHOULD BE KEPT IN MIND WHEN DRAWING THE FINAL BALANCE.

WE EXPRESS OUR GRATITUDE TO THE HUNGARIAN ACADEMY OF SCIENCES FOR FOUNDING AND FUNDING OF OUR JOURNAL AND FOR MAKING FOREIGN-LANGUAGE PUBLICATION POSSIBLE IN A DIFFICULT HISTORICAL PERIOD.

E. STARK M.D.
Editor-in-Chief

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*Lectures presented at the Meeting of the Korányi Sándor Society, Budapest, April 1994

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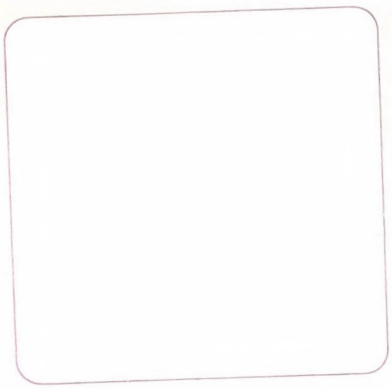

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Akadémiai Kiadó és Nyomda, Budapest

INFORMATION FOR AUTHORS

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Form of manuscripts

Two copies of the manuscript typewritten double-spaced with margins at least 4 cm wide should be submitted. Pages should be numbered consecutively. The first page should contain (1) the title of the paper (2) the initials and first name(s) of the author(s), (3) name of the institution where the work was done, (4) name and address of the author to whom correspondence and offprint requests should be addressed — this will appear as a footnote, (5) an abstract not exceeding 250 words which states the purposes of the study, the main findings and principal conclusions. Below the abstract provide 3 to 10 keywords that will assist indexers in cros-sindexing the article.

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Unusual abbreviations should be identified in an alphabetical list typed after the abstract and keywords.

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These should be cited in the text as numbers in square brackets. The list of references should contain in alphabetical order of the first authors' names the following: authors' last names with initials; for journal articles the title of the paper (lower case), journal title abbreviated according to the style used in Index Medicus, volume number, inclusive page numbers, year of publication in parentheses; for books the title (upper and lower case), publisher, place and date of publication. Only manuscripts accepted for publication may be included in the reference list.

Examples:

1. Stagg, B. H., Temperly, J. M., Wyllie, J. H.: The fate of pentagastrin. Gut **12,** 825–829 (1971)
2. Falkner, F.: Prevention in Childhood of Health Problems and Adult Life. WHO, Geneva 1980
3. Fishman, A. P.: Dynamics of pulmonary circulation. In: Hamilton, W. F., Dow, P. (eds): Handbook of Physiology. American Physiological Society, Washington 1963, pp. 65–79

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Figures should be submitted in duplicate. They must be numbered consecutively with arabic numerals. All figures should bear the name of the first author, the figure number and an arrow indicating the top. Cite each figure in the text and indicate its approximate place on the margin. If a figure has been published, acknowledge the original source and submit written permission from the copyright holder to reproduce the material. Figure capitions should be submitted typed double-spaced on a separete sheet.

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